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(54) **THYROGLOBULIN QUANTITATION BY MASS SPECTROMETRY**

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C12Q 1/00 (2006.01)

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USPC **436/86**; 436/171; 436/173; 435/6.19; 435/7.1; 435/23

(58) **Field of Classification Search**
USPC 436/86, 171, 173; 435/7, 7.1, 6.19, 435/23
See application file for complete search history.

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(57) **ABSTRACT**

Provided are methods for determining the amount of thyroglobulin in a sample using various purification steps followed by mass spectrometry. The methods generally involve purifying thyroglobulin in a test sample, digesting thyroglobulin to form peptide T129, purifying peptide T129, ionizing peptide T129, detecting the amount of peptide T129 ion generated, and relating the amount of peptide T129 ion to the amount of thyroglobulin originally present in the sample.

28 Claims, 15 Drawing Sheets

P01266 Sequence

MALVLEIFTLLASICWVSANIFEYQVDAQPLRPCELQRETAFLKQADYVPQCAEDGSFQT
VQCQNDGRSCWCVGANGSEVLGSRQPGRPVACLSFCQLQKQQILLSGYINSTDTSYLPQC
QDSGDYAPVQCDVQQVQCWCVDAEGMEVYGTRQLGRPKRCPRSCEIFNRRLLHGVGDKSP
PQCSAEGEPMPVQCKPVNTTDMMIFDLVHSYNRFPDAFVTFSSFQRRFPEVSGYCHCADS
QGRELAETGLELLLDEIYDTIFAGLDLPSTFTETTLYRILQRRFLAVQSVISGRFRCPTK
CEVERFTATSFGHPYVPSCRRNGDYQAVQCQTEGPCWCVDAQGKEMHGTRQQGEPPSCAE
GQSCASERQQALSRLYFGTSGYFSQHDLFSSPEKRWASPRVARFATSCPPTIKELFVDSG
LLRPMVEGQSQQFSVSENLLKEAIRAIFPSRGLARLALQFTTNPKRLQQNLFGGKFLVNV
GQFNLSGALGTRGTFNFSQFFQQLGLASFLNGGRQEDLAKPLSVGLDSNSSTGTPEAAKK
DGTMNKPTVGSFGFEINLQENQNALKFLASLLELPEFLLFLQHAISVPEDVARDLGDVME
TVLSSQTCEQTPERLFVPSCTTEGSYEDVQCFSGECWCVNSWGKELPGSRVRGGQPRCPT
DCEKQRARMQSLMGSQPAGSTLFVPACTSEGHFLPVQCFNSECYCVDAEGQAIPGTRSAI
GKPKKCPTPCQLQSEQAFLRTVQALLSNSSMLPTLSDTYIPQCSTDGQWRQVQCNGPPEQ
VFELYQRWEAQNKGQDLTPAKLLVKIMSYREAASGNFSLFIQSLYEAGQQDVFPVLSQYP
SLQDVPLAALEGKRPQPRENILLEPYLFWQILNGQLSQYPGSYSDFSTPLAHFDLRNCWC
VDEAGQELEGMRSEPSKLPTCPGSCEEAKLRVLQFIRETEEIVSASNSSRFPLGESFLVA
KGIRLRNEDLGLPPLFPPREAFAEQFLRGSDYAIRLAAQSTLSFYQRRPFSPDDSAGASA
LLRSGPYMPQCDAFGSWEPVQCHAGTGHCWCVDEKGGFIPGSLTARSLQIPQCPTTCEKS
RTSGLLSSWKQARSQENPSPKDLFVPACLETGEYARLQASGAGTWCVDPASGEELRPGSS
SSAQCPSLCNVLKSGVLSRRVSPGYVPACRAEDGGFSPVQCDQAQGSCWCVMDSGEEVPG
TRVTGGQPACESPRCPLPFNASEVVGGTILCETISGPTGSAMQQCQLLCRQGSWSVFPPG
PLICSLESGRWESQLPQPRACQRPQLWQTIQTQGHFQLQLPPGKMCSADYADLLQTFQVF
ILDELTARGFCQIQVKTFGTLVSIPVCNNSSVQVGCLTRERLGVNVTWKSRLEDIPVASL
PDLHDIERALVGKDLLGRFTDLIQSGSFQLHLDSKTFPAETIRFLQGDHFGTSPRTWFGC
SEGFYQVLTSEASQDGLGCVKCPEGSYSQDEECIPCPVGFYQEQAGSLACVPCPVGRTTI
SAGAFSQTHCVTDCQRNEAGLQCDQNGQYRASQKDRGSGKAFCVDGEGRRLPWWETEAPL
EDSQCLMMQKFEKVPESKVIFDANAPVAVRSKVPDSEFPVMQCLTDCTEDEACSFFTVST
TEPEISCDFYAWTSDNVACMTSDQKRDALGNSKATSFGSLRCQVKVRSHGQDSPAVYLKK
GQGSTTTLQKRFEPTGFQNMLSGLYNPIVFSASGANLTDAHLFCLLACDRDLCCDGFVLT
QVQGGAIICGLLSSPSVLLCNVKDWMDPSEAWANATCPGVTYDQESHQVILRLGDQEFIK
SLTPLEGTQDTFTNFQQVYLWKDSDMGSRPESMGCRKDTVPRPASPTEAGLTTELPSPVD
LNQVIVNGNQSLSSQKHWLFKHLFSAQQANLWCLSRCVQEHSFCQLAEITESASLYFTCT
LYPEAQVCDDIMESNAQGCRLILPQMPKALFRKKVILEDKVKNFYTRLPFQKLMGISIRN
KVPMSEKSISNGFFECERRCDADPCCTGFGFLNVSQLKGGEVTCLTLNSLGIQMCSEENG
GAWRILDCGSPDIEVHTYPFGWYQKPIAQNNAPSFCPLVVLPSLTEKVSLDSWQSLALSS
VVVDPSIRHFDVAHVSTAATSNFSAVRDLCLSECSQHEACLITTLQTQPGAVRCMFYADT
QSCTHSLQGQNCRLLLREEATHIYRKPGISLLSYEASVPSVPISTHGRLLGRSQAIQVGT
SWKQVDQFLGVPYAAPPLAERRFQAPEPLNWTGSWDASKPRASCWQPGTRTSTSPGVSED
CLYLNVFIPQNVAPNASVLVFFHNTMDREESEGWPAIDGSFLAAVGNLIVVTASYRVGVF
GFLSSGSGEVSGNWGLLDQVAALTWVQTHIRGFGGDPRRVSLAADRGGADVASIHLLTAR
ATNSQLFRRAVLMGGSALSPAAVISHERAQQQAIALAKEVSCPMSSSQEVVSCLRQKPAN
VLNDAQTKLLAVSGPFHYWGPVIDGHFLREPPARALKRSLWVEVDLLIGSSQDDGLINRA
KAVKQFEESRGRTSSKTAFYQALQNSLGGEDSDARVEAAATWYYSLEHSTDDYASFSRAL
ENATRDYFIICPIIDMASAWAKRARGNVFMYHAPENYGHGSLELLADVQFALGLPFYPAY
EGQFSLEEKSLSLKIMQYFSHFIRSGNPNYPYEPSRKVPTFATPWPDFVPRAGGENYKEF
SELLPNRQGLKKADCSFWSKYISSLKTSADGAKGGQSAESEEEELTAGSGLREDLLSLQE
PGSKTYSK

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Figure 1

P01266 Sequence

MALVLEIFTLLASICWVSANIFEYQVDAQPLRPCELQRETAFLKQADYVPQCAEDGSFQT
VQCQNDGRSCWCVGANGSEVLGSRQPGRPVACLSFCQLQKQQILLSGYINSTDTSYLPQC
QDSGDYAPVQCDVQQVQCWCVDAEGMEVYGTRQLGRPKRCPRSCEIRNRRLLHGVGDKSP
PQCSAEGEFMPVQCKFVNTTDMMIFDLVHSYNRFPDAFVTFSSFQRRFPEVSGYCHCADS
QGRELAETGLELLLDEIYDTIFAGLDLPSTFTETTLYRILQRRFLAVQSVISGRFRCPTK
CEVERFTATSFGHPYVPSCRRNGDYQAVQCQTEGPCWCVDAQGKEMHGTRQQGEPPSCAE
GQSCASERQQALSRLYFGTSGYFSQHDLFSSPEKRWASPRVARFATSCPPTIKELFVDSG
LLRPMVEGQSQQFSVSENLLKEAIRAIFPSRGLARLALQFTTNPKRLQQNLFGGKFLVNV
GQFNLSGALGTRGTFNFSQFFQQLGLASFLNGGRQEDLAKPLSVGLDSNSSTGTPEAAKK
DGTMNKPTVGSFGFEINLQENQNALKFLASLLELPEFLLFLQHAISVPEDVARDLGDVME
TVLSSQTCEQTPERLFVPSCTTEGSYEDVQCFSGECWCVNSWGKELPGSRVRGGQPRCPT
DCEKQRARMQSLMGSQPAGSTLFVPACTSEGHFLPVQCFNSECYCVDAEGQAIPGTRSAI
GKPKKCPTPCQLQSEQAFLRTVQALLSNSSMLPTLSDTYIPQCSTDGQWRQVQCNGPPEQ
VFELYQRWEAQNKGQDLTPAKLLVKIMSYREAASGNFSLFIQSLYEAGQQDVFPVLSQYP
SLQDVPLAALEGKRPQPRENILLEPYLFWQILNGQLSQYPGSYSDFSTPLAHFDLRNCWC
VDEAGQELEGMRSEPSKLPTCPGSCEEAKLRVLQFIRETEEIVSASNSSRFPLGESFLVA
KGIRLRNEDLGLPPLFPPREAFAEQFLRGSDYAIRLAAQSTLSFYQRRRFSPDDSAGASA
LLRSGPYMPQCDAFGSWEPVQCHAGTGHCWCVDEKGGFIPGSLTARSLQIPQCPTTCEKS
RTSGLLSSWKQARSQENPSPKDLFVPACLETGEYARLQASGAGTWCVDPASGEELRPGSS
SSAQCPSLCNVLKSGVLSRRVSPGYVPACRAEDGGFSPVQCDQAQGSCWCVMDSGEEVPG
TRVTGGQPACESPRCPLPFNASEVVGGTILCETISGPTGSAMQQCQLLCRQGSWSVFPPG
PLICSLESGRWESQLPQPRACQRPQLWQTIQTQGHFQLQLPPGKMCSADYADLLQTFQVF
ILDELTARGFCQIQVKTFGTLVSIPVCNNSSVQVGCLTRERLGVNVTWKSRLEDIPVASL
PDLHDIERALVGKDLLGRFTDLIQSGSFQLHLDSKTFPAETIRFLQGDHFGTSPRTWFGC
SEGFYQVLTSEASQDGLGCVKCPEGSYSQDEECIPCPVGFYQEQAGSLACVPCPVGRTTI
SAGAFSQTHCVTDCQRNEAGLQCDQNGQYRASQKDRGSGKAFCVDGEGRRLPWWETEAPL
EDSQCLMMQKFEKVPESKVIFDANAPVAVRSKVPDSEFPVMQCLTDCTEDEACSFFTVST
TEPEISCDFYAWTSDNVACMTSDQKRDALGNSKATSFGSLRCQVKVRSHGQDSPAVYLKK
GQGSTTTLQKRFEPTGFQNMLSGLYNPIVFSASGANLTDAHLFCLLACDRDLCCDGFVLT
QVQGGAIICGLLSSPSVLLCNVKDWMDPSEAWANATCPGVTYDQESHQVILRLGDQEFIK
SLTPLEGTQDTFTNFQQVYLWKDSDMGSRPESMGCRKDTVPRPASPTEAGLTTELFSPVD
LNQVIVNGNQSLSSQKHWLFKHLFSAQQANLWCLSRCVQEHSFCQLAEITESASLYFTCT
LYPEAQVCDDIMESNAQGCRLILPQMPKALFRKKVILEDKVKNFYTRLPFQKLMGISIRN
KVPMSEKSISNGFFECERRCDADPCCTGFGFLNVSQLKGGEVTCLTLNSLGIQMCSEENG
GAWRILDCGSPDIEVHTYPFGWYQKPIAQNNAPSFCPLVVLPSLTEKVSLDSWQSLALSS
VVVDPSIRHFDVAHVSTAATSNFSAVRDLCLSECSQHEACLITTLQTQPGAVRCMFYADT
QSCTHSLQGQNCRLLLREEATHIYRKPGISLLSYEASVPSVPISTHGRLLGRSQAIQVGT
SWKQVDQFLGVPYAAPPLAERRFQAPEPLNWTGSWDASKPRASCWQPGTRTSTSPGVSED
CLYLNVFIPQNVAPNASVLVFFHNTMDREESEGWPAIDGSFLAAVGNLIVVTASYRVGVF
GFLSSGSGEVSGNWGLLDQVAALTWVQTHIRGFGGDPRRVSLAADRGGADVASIHLLTAR
ATNSQLFRRAVLMGGSALSPAAVISHERAQQQAIALAKEVSCPMSSSQEVVSCLRQKPAN
VLNDAQTKLLAVSGPFHYWGPVIDGHFLREPPARALKRSLWVEVDLLIGSSQDDGLINRA
KAVKQFEESRGRTSSKTAFYQALQNSLGGEDSDARVEAAATWYYSLEHSTDDYASFSRAL
ENATRDYFIICPIIDMASAWAKRARGNVFMYHAPENYGHGSLELLADVQFALGLPFYPAY
EGQFSLEEKSLSLKIMQYFSHFIRSGNPNYPYEFSRKVPTFATPWPDFVPRAGGENYKEF
SELLPNRQGLKKADCSFWSKYISSLKTSADGAKGGQSAESEEEELTAGSGLREDLLSLQE
PGSKTYSK

Figure 2

P01266-2 Isoform 2 Sequence

>sp_vs|P01266-2|THYG_HUMAN Isoform 2 of P01266 - Homo sapiens (Human)
MALVLEIFTLLASICWVSANIFEYQVDAQPLRPCELQRETAFLKQADYVPQCAEDGSFQT
VQCQNDGRSCWCVGANGSEVLGSRQPGRPVACLSFCQLQKQQILLSGYINSTDTSYLPQC
QDSGDYAPVQCDVQQVQCWCVDAEGMEVYGTRQLGRPKRCPRSCEIRNRRLLHGVGDKSP
PQCSAEGEFMPVQCKFVNTTDMMIFDLVHSYNRFPDAFVTFSSFQRRFPEVSGYCHCADS
QGRELAETGLELLLDEIYDTIFAGLDLPSTFTETTLYRILQRRFLAVQSVISGRFRCPTK
CEVERFTATSFGHPYVPSCRRNGDYQAVQCQTEGPCWCVDAQGKEMHGTRQQGEPPSCAE
GQSCASERQQALSRLYFGTSGYFSQHDLFSSPEKRWASPRVARFATSCPPTIKELFVDSG
LLRPMVEGQSQQFSVSENLLKEAIRAIFPSRGLARLALQFTTNPKRLQQNLFGGKFLVNV
GQFNLSGALGTRGTFNFSQFFQQLGLASFLNGGRQEDLAKPLSVGLDSNSSTGTPEAAKK
DGTMNKPTVGSFGFEINLQENQNALKFLASLLELPEFLLFLQHAISVPEDVARDLGDVME
TVLSSQTCEQTPERLFVPSCTTEGSYEDVQCFSGECWCVNSWGKELPGSRVRGGQPRCPT
DCEKQRARMQSLMGSQPAGSTLFVPACTSEGHFLPVQCFNSECYCVDAEGQAIPGTRSAI
GKPKKCPTPCQLQSEQAFLRTVQALLSNSSMLPTLSDTYIPQCSTDGQWRQVQCNGPPEQ
VFELYQRWEAQNKGQDLTPAKLLVKIMSYREAASGNFSLFIQSLYEAGQQDVFPVLSQYP
SLQDVPLAALEGKRPQPRENILLEPYLFWQILNGQLSQYPGSYSDFSTPLAHFDLRNCWC
VDEAGQELEGMRSEPSKLPTCPGSCEEAKLRVLQFIRETEEIVSASNSSRFPLGESFLVA
KGIRLRNEDLGLPPLFPPREAFAEQFLRGSDYAIRLAAQSTLSFYQRRRFSPDDSAGASA
LLRSGPYMPQCDAFGSWEPVQCHAGTGHCWCVDEKGGFIPGSLTARSLQIPQCPTTCEKS
RTSGLLSSWKQARSQENPSPKDLFVPACLETGEYARLQASGAGTWCVDPASGEELRPGSS
SSAQCPSLCNVLKSGVLSRRVSPGYVPACRAEDGGFSPVQCDQAQGSCWCVMDSGEEVPG
TRVTGGQPACESPRCPLPFNASEVVGGTILCETISGPTGSAMQQCQLLCRQGSWSVFPPG
PLICSLESGRWESQLPQPRACQRPQLWQTIQTQGHFQLQLPPGKMCSADYADLLQTFQVF
ILDELTARGFCQIQVKTFGTLVSIPVCNNSSVQVGCLTRERLGVNVTWKSRLEDIPVASL
PDLHDIERALVGKDLLGRFTDLIQSGSFQLHLDSKTFPAETIRFLQGDHFGTSPRTWFGC
SEGFYQVLTSEASQDGLGCVKCPEGSYSQDEECIPCPVGFYQEQAGSLACVPCPVGRTTI
SAGAFSQTHLMQKFEKVPESKVIFDANAPVAVRSKVPDSEFPVMQCLTDCTEDEACSFFT
VSTTEPEISCDFYAWTSDNVACMTSDQKRDALGNSKATSFGSLRCQVKVRSHGQDSPAVY
LKKGQGSTTTLQKRFEPTGFQNMLSGLYNPIVFSASGANLTDAHLFCLLACDRDLCCDGF
VLTQVQGGAIICGLLSSPSVLLCNVKDWMDPSEAWANATCPGVTYDQESHQVILRLGDQE
FIKSLTPLEGTQDTFTNFQQVYLWKDSDMGSRPESMGCRKDTVPRPASPTEAGLTTELFS
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IRNKVPMSEKSISNGFFECERRCDADPCCTGFGFLNVSQLKGGEVTCLTLNSLGIQMCSE
ENGGAWRILDCGSPDIEVHTYPFGWYQKPIAQNNAPSFCPLVVLPSLTEKVSLDSWQSLA
LSSVVVDPSIRHFDVAHVSTAATSNFSAVRDLCLSECSQHEACLITTLQTQPGAVRCMFY
ADTQSCTHSLQGQNCRLLLREEATHIYRKPGISLLSYEASVPSVPISTHGRLLGRSQAIQ
VGTSWKQVDQFLGVPYAAPPLAERRFQAPEPLNWTGSWDASKPRASCWQPGTRTSTSPGV
SEDCLYLNVFIPQNVAPNASVLVFFHNTMDREESEGWPAIDGSFLAAVGNLIVVTASYRV
GVFGFLSSGSGEVSGNWGLLDQVAALTWVQTHIRGFGGDPRRVSLAADRGGADVASIHLL
TARATNSQLFRRAVLMGGSALSPAAVISHERAQQQAIALAKEVSCPMSSSQEVVSCLRQK
PANVLNDAQTKLLAVSGPFHYWGPVIDGHFLREPPARALKRSLWVEVDLLIGSSQDDGLI
NRAKAVKQFEESRGRTSSKTAFYQALQNSLGGEDSDARVEAAATWYYSLEHSTDDYASFS
RALENATRDYFIICPIIDMASAWAKRARGNVFMYHAPENYGHGSLELLADVQFALGLPFY
PAYEGQFSLEEKSLSLKIMQYFSHFIRSGNPNYPYEFSRKVPTFATPWPDFVPRAGGENY
KEFSELLPNRQGLKKADCSFWSKYISSLKTSADGAKGGQSAESEEEELTAGSGLREDLLS
LQEPGSKTYSK

Figure 3

Q59GF0 (Tg variant-Fragment) Sequence

>Q59GF0|Q59GF0_HUMAN Thyroglobulin variant (Fragment) - Homo sapiens (Human).

IPRKPISKRPVRPSLPRSPRCPLPFNASEVVGGTILCETISGPTGSAMQQCQLLCRQGSW
SVFPPGPLICSLESGRWESQLPQPRACQRPQLWQTIQTQGHFQLQLPPGKMCSADYAGLL
QTFQVFILDELTARGFCQIQVKTFGTLVSIPVCNNSSVQVGCLTRERLGVNVTWKSRLED
IPVASLPDLHDIERALVGKDLLGRFTDLIQSGSFQLHLDSKTFPAETIRFLQGDHFGTSP
RTWFGCSEGFYQVLTSEASQDGLGCVKCPEGSYSQDEECIPCPVGFYQEQAGSLACVPCP
VGRTTISAGAFSQTHCVTDCQRNEAGLQCDQNGQYRASQKDRGSGKAFCVDGEGRRLPWW
ETEAPLEDSQCLMMQKFEKVPESKVIFDANAPVAVRSKVPDSEFPVMQCLTDCTEDEACS
FFTVSTTEPEISCDFYAWTSDNVACMTSDQKRDALGNSKATSFGSLRCQVKVRSHGQDSP
AVYLKKGQGSTTTLQKRFEPTGFQNMLSGLYNPIVFSASGANLTDAHLFCLLACDRDLCC
DGFVLTQVQGGAIICGLLSSPSVLLCNVKDWMDPSEAWANATCPGVTYDQESHQVILRLG
DQEFIKSLTPLEGTQDTFTNFQQVYLWKDSDMGSRPESMGCRKNTVPRPASPTEAGLTTE
LFSPVDLNQVIVNGNQSLSSQKHWLFKHLFSAQQANLWCLSRCVQEHSFCQLAEITESAS
LYFTCTLYPEAQVCDDIMESNAQGCRLILPQMPKALFRKKVILEDKVKNFYTRLPFQKLT
GISIRNKVPMSEKSISNGFFECERRCDADPCCTGFGFLNVSQLKGGEVTCLTLNSLGIQM
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SLALSSVVVDPSIRHFDVAHVSTAATSNFSAVRDLCLSECSQHEACLITTLQTQPGAVRC
MFYADTQSCTHSLQGQNCRLLLREEATHIYRKPGISLLSYEASVPSVPISTHGRLLGRSQ
AIQVGTSWKQVDQFLGVPYAAPPLAERRFQAPEPLNWTGSWDASKPRASCWQPGTRTSTS
PGVSEDCLYLNVFIPQNVAPNASVLVFFHNTMDREESEGWPAIDGSFLAAVGNLIVVTAS
YRVGVFGFLSSGSGEVSGNWGLLDQVAALTWVQTHIRGFGGDPRRVSLAADRGGADVASI
HLLTARATNSQLFRRAVLMGGSALSPAAVISHERAQQQAIALAKEVSCPMSSSQEVVSCL
RQKPANVLNDAQTKLLAVSGPFHYWGPVIDGHFLREPPARALKRSLWVEVDLLIGSSQDD
GLINRAKAVKQFEESQGRTSSKTAFYQALQNSLGGEDSDARVEAAATWYYSLEHSTDDYA
SFSRALENATRDYFIICPIIDMASAWAKRARGNVFMYHAPENYGHGSLELLADVQFALGL
PFYPAYEGQFSLEEKSLSLKIMQYFSHFIRSGNPNYPYEFSRKVPTFATPWPDFVPRAGG
ENYKEFSELLPNRQGLKKADCSFWSKYISSLKTSADGAKGGQSAESEEEELTAGSGLRED
LLSLQEPGSKTYSK

Figure 4 (1 of 7)

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          10         20         30         40         50         60
MALVLEIFTL LASICWVSAN IFEYQVDAQP LRPCELQRET AFLKQADYVP QCAEDGSFQT
MALVLEIFTL LASICWVSAN IFEYQVDAQP LRPCELQRET AFLKQADYVP QCAEDGSFQT

          70         80         90        100        110        120
VQCQNDGRSC WCVGANGSEV LGSRQPGRPV ACLSFCQLQK QQILLSGYIN STDTSYLPQC
VQCQNDGRSC WCVGANGSEV LGSRQPGRPV ACLSFCQLQK QQILLSGYIN STDTSYLPQC

         130        140        150        160        170        180
QDSGDYAPVQ CDVQQVQCWC VDAEGMEVYG TRQLGRPKRC PRSCEIRNRR LLHGVGDKSP
QDSGDYAPVQ CDVQQVQCWC VDAEGMEVYG TRQLGRPKRC PRSCEIRNRR LLHGVGDKSP

         190        200        210        220        230        240
PQCSAEGEFM PVQCKFVNTT DMMIFDLVHS YNRFPDAFVT FSSFQRRFPE VSGYCHCADS
PQCSAEGEFM PVQCKFVNTT DMMIFDLVHS YNRFPDAFVT FSSFQRRFPE VSGYCHCADS

         250        260        270        280        290        300
QGRELAETGL ELLLDEIYDT IFAGLDLPST FTETTLYRIL QRRFLAVQSV ISGRFRCPTK
QGRELAETGL ELLLDEIYDT IFAGLDLPST FTETTLYRIL QRRFLAVQSV ISGRFRCPTK

         310        320        330        340        350        360
CEVERFTATS FGHPYVPSCR RNGDYQAVQC QTEGPCWCVD AQGKEMHGTR QQGEPPSCAE
CEVERFTATS FGHPYVPSCR RNGDYQAVQC QTEGPCWCVD AQGKEMHGTR QQGEPPSCAE

         370        380        390        400        410        420
GQSCASERQQ ALSRLYFGTS GYFSQHDLFS SPEKRWASPR VARFATSCPP TIKELFVDSG
GQSCASERQQ ALSRLYFGTS GYFSQHDLFS SPEKRWASPR VARFATSCPP TIKELFVDSG

         430        440        450        460        470        480
LLRPMVEGQS QQFSVSENLL KEAIRAIFPS RGLARLALQF TTNPKRLQQN LFGGKFLVNV
LLRPMVEGQS QQFSVSENLL KEAIRAIFPS RGLARLALQF TTNPKRLQQN LFGGKFLVNV
```

Figure 4 (2 of 7)

```
          490         500         510         520         530         540
GQFNLSGALG TRGTFNFSQF FQQLGLASFL NGGRQEDLAK PLSVGLDSNS STGTPEAAKK
GQFNLSGALG TRGTFNFSQF FQQLGLASFL NGGRQEDLAK PLSVGLDSNS STGTPEAAKK

          550         560         570         580         590         600
DGTMNKPTVG SFGFEINLQE NQNALKFLAS LLELPEFLLF LQHAISVPED VARDLGDVME
DGTMNKPTVG SFGFEINLQE NQNALKFLAS LLELPEFLLF LQHAISVPED VARDLGDVME

          610         620         630         640         650         660
TVLSSQTCEQ TPERLFVPSC TTEGSYEDVQ CFSGECWCVN SWGKELPGSR VRGGQPRCPT
TVLSSQTCEQ TPERLFVPSC TTEGSYEDVQ CFSGECWCVN SWGKELPGSR VRGGQPRCPT

          670         680         690         700         710         720
DCEKQRARMQ SLMGSQPAGS TLFVPACTSE GHFLPVQCFN SECYCVDAEG QAIPGTRSAI
DCEKQRARMQ SLMGSQPAGS TLFVPACTSE GHFLPVQCFN SECYCVDAEG QAIPGTRSAI

          730         740         750         760         770         780
GKPKKCPTPC QLQSEQAFLR TVQALLSNSS MLPTLSDTYI PQCSTDGQWR QVQCNGPPEQ
GKPKKCPTPC QLQSEQAFLR TVQALLSNSS MLPTLSDTYI PQCSTDGQWR QVQCNGPPEQ

          790         800         810         820         830         840
VFELYQRWEA QNKGQDLTPA KLLVKIMSYR EAASGNFSLF IQSLYEAGQQ DVFPVLSQYP
VFELYQRWEA QNKGQDLTPA KLLVKIMSYR EAASGNFSLF IQSLYEAGQQ DVFPVLSQYP

          850         860         870         880         890         900
SLQDVPLAAL EGKRPQPREN ILLEPYLFWQ ILNGQLSQYP GSYSDFSTPL AHFDLRNCWC
SLQDVPLAAL EGKRPQPREN ILLEPYLFWQ ILNGQLSQYP GSYSDFSTPL AHFDLRNCWC

          910         920         930         940         950         960
VDEAGQELEG MRSEPSKLPT CPGSCEEAKL RVLQFIRETE EIVSASNSSR FPLGESFLVA
VDEAGQELEG MRSEPSKLPT CPGSCEEAKL RVLQFIRETE EIVSASNSSR FPLGESFLVA
```

Figure 4 (3 of 7)

```
       970        980        990       1000       1010       1020
KGIRLRNEDL GLPPLFPPRE AFAEQFLRGS DYAIRLAAQS TLSFYQRRRF SPDDSAGASA
KGIRLRNEDL GLPPLFPPRE AFAEQFLRGS DYAIRLAAQS TLSFYQRRRF SPDDSAGASA

      1030       1040       1050       1060       1070       1080
LLRSGPYMPQ CDAFGSWEPV QCHAGTGHCW CVDEKGGFIP GSLTARSLQI PQCPTTCEKS
LLRSGPYMPQ CDAFGSWEPV QCHAGTGHCW CVDEKGGFIP GSLTARSLQI PQCPTTCEKS

      1090       1100       1110       1120       1130       1140
RTSGLLSSWK QARSQENPSP KDLFVPACLE TGEYARLQAS GAGTWCVDPA SGEELRPGSS
RTSGLLSSWK QARSQENPSP KDLFVPACLE TGEYARLQAS GAGTWCVDPA SGEELRPGSS

      1150       1160       1170       1180       1190       1200
SSAQCPSLCN VLKSGVLSRR VSPGYVPACR AEDGGFSPVQ CDQAQGSCWC VMDSGEEVPG
SSAQCPSLCN VLKSGVLSRR VSPGYVPACR AEDGGFSPVQ CDQAQGSCWC VMDSGEEVPG
                                                          IPRKPI

      1210       1220       1230       1240       1250       1260
TRVTGGQPAC ESPRCPLPFN ASEVVGGTIL CETISGPTGS AMQQCQLLCR QGSWSVFPPG
TRVTGGQPAC ESPRCPLPFN ASEVVGGTIL CETISGPTGS AMQQCQLLCR QGSWSVFPPG
SKRPVRPSLP RSPRCPLPFN ASEVVGGTIL CETISGPTGS AMQQCQLLCR QGSWSVFPPG

      1270       1280       1290       1300       1310       1320
PLICSLESGR WESQLPQPRA CQRPQLWQTI QTQGHFQLQL PPGKMCSADY ADLLQTFQVF
PLICSLESGR WESQLPQPRA CQRPQLWQTI QTQGHFQLQL PPGKMCSADY ADLLQTFQVF
PLICSLESGR WESQLPQPRA CQRPQLWQTI QTQGHFQLQL PPGKMCSADY AGLLQTFQVF

      1330       1340       1350       1360       1370       1380
ILDELTARGF CQIQVKTFGT LVSIPVCNNS SVQVGCLTRE RLGVNVTWKS RLEDIPVASL
ILDELTARGF CQIQVKTFGT LVSIPVCNNS SVQVGCLTRE RLGVNVTWKS RLEDIPVASL
ILDELTARGF CQIQVKTFGT LVSIPVCNNS SVQVGCLTRE RLGVNVTWKS RLEDIPVASL
```

Figure 4 (4 of 7)

```
      1390        1400        1410        1420        1430        1440
PDLHDIERAL VGKDLLGRFT DLIQSGSFQL HLDSKTFPAE TIRFLQGDHF GTSPRTWFGC
PDLHDIERAL VGKDLLGRFT DLIQSGSFQL HLDSKTFPAE TIRFLQGDHF GTSPRTWFGC
PDLHDIERAL VGKDLLGRFT DLIQSGSFQL HLDSKTFPAE TIRFLQGDHF GTSPRTWFGC

      1450        1460        1470        1480        1490        1500
SEGFYQVLTS EASQDGLGCV KCPEGSYSQD EECIPCPVGF YQEQAGSLAC VPCPVGRTTI
SEGFYQVLTS EASQDGLGCV KCPEGSYSQD EECIPCPVGF YQEQAGSLAC VPCPVGRTTI
SEGFYQVLTS EASQDGLGCV KCPEGSYSQD EECIPCPVGF YQEQAGSLAC VPCPVGRTTI

      1510        1520        1530        1540        1550        1560
SAGAFSQTHC VTDCQRNEAG LQCDQNGQYR ASQKDRGSGK AFCVDGEGRR LPWWETEAPL
SAGAFSQTHL
SAGAFSQTHC VTDCQRNEAG LQCDQNGQYR ASQKDRGSGK AFCVDGEGRR LPWWETEAPL

      1570        1580        1590        1600        1610        1620
EDSQCLMMQK FEKVPESKVI FDANAPVAVR SKVPDSEFPV MQCLTDCTED EACSFFTVST
   MQK FEKVPESKVI FDANAPVAVR SKVPDSEFPV MQCLTDCTED EACSFFTVST
EDSQCLMMQK FEKVPESKVI FDANAPVAVR SKVPDSEFPV MQCLTDCTED EACSFFTVST

      1630        1640        1650        1660        1670        1680
TEPEISCDFY AWTSDNVACM TSDQKRDALG NSKATSFGSL RCQVKVRSHG QDSPAVYLKK
TEPEISCDFY AWTSDNVACM TSDQKRDALG NSKATSFGSL RCQVKVRSHG QDSPAVYLKK
TEPEISCDFY AWTSDNVACM TSDQKRDALG NSKATSFGSL RCQVKVRSHG QDSPAVYLKK

      1690        1700        1710        1720        1730        1740
GQGSTTTLQK RFEPTGFQNM LSGLYNPIVF SASGANLTDA HLFCLLACDR DLCCDGFVLT
GQGSTTTLQK RFEPTGFQNM LSGLYNPIVF SASGANLTDA HLFCLLACDR DLCCDGFVLT
GQGSTTTLQK RFEPTGFQNM LSGLYNPIVF SASGANLTDA HLFCLLACDR DLCCDGFVLT

      1750        1760        1770        1780        1790        1800
QVQGGAIICG LLSSPSVLLC NVKDWMDPSE AWANATCPGV TYDQESHQVI LRLGDQEFIK
QVQGGAIICG LLSSPSVLLC NVKDWMDPSE AWANATCPGV TYDQESHQVI LRLGDQEFIK
QVQGGAIICG LLSSPSVLLC NVKDWMDPSE AWANATCPGV TYDQESHQVI LRLGDQEFIK
```

Figure 4 (5 of 7)

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        1810        1820        1830        1840        1850        1860
SLTPLEGTQD TFTNFQQVYL WKDSDMGSRP ESMGCRKDTV PRPASPTEAG LTTELFSPVD
SLTPLEGTQD TFTNFQQVYL WKDSDMGSRP ESMGCRKNTV PRPASPTEAG LTTELFSPVD
        1870        1880        1890        1900        1910        1920
LNQVIVNGNQ SLSSQKHWLF KHLFSAQQAN LWCLSRCVQE HSFCQLAEIT ESASLYFTCT
LNQVIVNGNQ SLSSQKHWLF KHLFSAQQAN LWCLSRCVQE HSFCQLAEIT ESASLYFTCT
LNQVIVNGNQ SLSSQKHWLF KHLFSAQQAN LWCLSRCVQE HSFCQLAEIT ESASLYFTCT
        1930        1940        1950        1960        1970        1980
LYPEAQVCDD IMESNAQGCR LILPQMPKAL FRKKVILEDK VKNFYTRLPF QKLMGISIRN
LYPEAQVCDD IMESNAQGCR LILPQMPKAL FRKKVILEDK VKNFYTRLPF QKLMGISIRN
LYPEAQVCDD IMESNAQGCR LILPQMPKAL FRKKVILEDK VKNFYTRLPF QKLTGISIRN
        1990        2000        2010        2020        2030        2040
KVPMSEKSIS NGFFECERRC DADPCCTGFG FLNVSQLKGG EVTCLTLNSL GIQMCSEENG
KVPMSEKSIS NGFFECERRC DADPCCTGFG FLNVSQLKGG EVTCLTLNSL GIQMCSEENG
KVPMSEKSIS NGFFECERRC DADPCCTGFG FLNVSQLKGG EVTCLTLNSL GIQMCSEENG
        2050        2060        2070        2080        2090        2100
GAWRILDCGS PDIEVHTYPF GWYQKPIAQN NAPSFCPLVV LPSLTEKVSL DSWQSLALSS
GAWRILDCGS PDIEVHTYPF GWYQKPIAQN NAPSFCPLVV LPSLTEKVSL DSWQSLALSS
GAWRILDCGS PDIEVHTYPF GWYQKPIAQN NAPSFCPLVV LPSLTEKVSL DSWQSLALSS
        2110        2120        2130        2140        2150        2160
VVVDPSIRHF DVAHVSTAAT SNFSAVRDLC LSECSQHEAC LITTLQTQPG AVRCMFYADT
VVVDPSIRHF DVAHVSTAAT SNFSAVRDLC LSECSQHEAC LITTLQTQPG AVRCMFYADT
VVVDPSIRHF DVAHVSTAAT SNFSAVRDLC LSECSQHEAC LITTLQTQPG AVRCMFYADT
```

Figure 4 (6 of 7)

```
          2170        2180        2190        2200        2210        2220
QSCTHSLQGQ NCRLLLREEA THIYRKPGIS LLSYEASVPS VPISTHGRLL GRSQAIQVGT
QSCTHSLQGQ NCRLLLREEA THIYRKPGIS LLSYEASVPS VPISTHGRLL GRSQAIQVGT
          2230        2240        2250        2260        2270        2280
SWKQVDQFLG VPYAAPPLAE RRFQAPEPLN WTGSWDASKP RASCWQPGTR TSTSPGVSED
SWKQVDQFLG VPYAAPPLAE RRFQAPEPLN WTGSWDASKP RASCWQPGTR TSTSPGVSED
SWKQVDQFLG VPYAAPPLAE RRFQAPEPLN WTGSWDASKP RASCWQPGTR TSTSPGVSED
          2290        2300        2310        2320        2330        2340
CLYLNVFIPQ NVAPNASVLV FFHNTMDREE SEGWPAIDGS FLAAVGNLIV VTASYRVGVF
CLYLNVFIPQ NVAPNASVLV FFHNTMDREE SEGWPAIDGS FLAAVGNLIV VTASYRVGVF
CLYLNVFIPQ NVAPNASVLV FFHNTMDREE SEGWPAIDGS FLAAVGNLIV VTASYRVGVF
          2350        2360        2370        2380        2390        2400
GFLSSGSGEV SGNWGLLDQV AALTWVQTHI RGFGGDPRRV SLAADRGGAD VASIHLLTAR
GFLSSGSGEV SGNWGLLDQV AALTWVQTHI RGFGGDPRRV SLAADRGGAD VASIHLLTAR
GFLSSGSGEV SGNWGLLDQV AALTWVQTHI RGFGGDPRRV SLAADRGGAD VASIHLLTAR
          2410        2420        2430        2440        2450        2460
ATNSQLFRRA VLMGGSALSP AAVISHERAQ QQAIALAKEV SCPMSSSQEV VSCLRQKPAN
ATNSQLFRRA VLMGGSALSP AAVISHERAQ QQAIALAKEV SCPMSSSQEV VSCLRQKPAN
ATNSQLFRRA VLMGGSALSP AAVISHERAQ QQAIALAKEV SCPMSSSQEV VSCLRQKPAN
          2470        2480        2490        2500        2510        2520
VLNDAQTKLL AVSGPFHYWG PVIDGHFLRE PPARALKRSL WVEVDLLIGS SQDDGLINRA
VLNDAQTKLL AVSGPFHYWG PVIDGHFLRE PPARALKRSL WVEVDLLIGS SQDDGLINRA
VLNDAQTKLL AVSGPFHYWG PVIDGHFLRE PPARALKRSL WVEVDLLIGS SQDDGLINRA
```

Figure 4 (7 of 7)

```
      2530        2540        2550        2560        2570        2580
KAVKQFEESR GRTSSKTAFY QALQNSLGGE DSDARVEAAA TWYYSLEHST DDYASFSRAL
KAVKQFEESR GRTSSKTAFY QALQNSLGGE DSDARVEAAA TWYYSLEHST DDYASFSRAL
KAVKQFEESQ GRTSSKTAFY QALQNSLGGE DSDARVEAAA TWYYSLEHST DDYASFSRAL
      2590        2600        2610        2620        2630        2640
ENATRDYFII CPIIDMASAW AKRARGNVFM YHAPENYGHG SLELLADVQF ALGLPFYPAY
ENATRDYFII CPIIDMASAW AKRARGNVFM YHAPENYGHG SLELLADVQF ALGLPFYPAY
ENATRDYFII CPIIDMASAW AKRARGNVFM YHAPENYGHG SLELLADVQF ALGLPFYPAY
      2650        2660        2670        2680        2690        2700
EGQFSLEEKS LSLKIMQYFS HFIRSGNPNY PYEFSRKVPT FATPWPDFVP RAGGENYKEF
EGQFSLEEKS LSLKIMQYFS HFIRSGNPNY PYEFSRKVPT FATPWPDFVP RAGGENYKEF
EGQFSLEEKS LSLKIMQYFS HFIRSGNPNY PYEFSRKVPT FATPWPDFVP RAGGENYKEF
      2710        2720        2730        2740        2750        2760
SELLPNRQGL KKADCSFWSK YISSLKTSAD GAKGGQSAES EEEELTAGSG LREDLLSLQE
SELLPNRQGL KKADCSFWSK YISSLKTSAD GAKGGQSAES EEEELTAGSG LREDLLSLQE
SELLPNRQGL KKADCSFWSK YISSLKTSAD GAKGGQSAES EEEELTAGSG LREDLLSLQE

PGSKTYSK
PGSKTYSK
PGSKTYSK
```

Figure 5

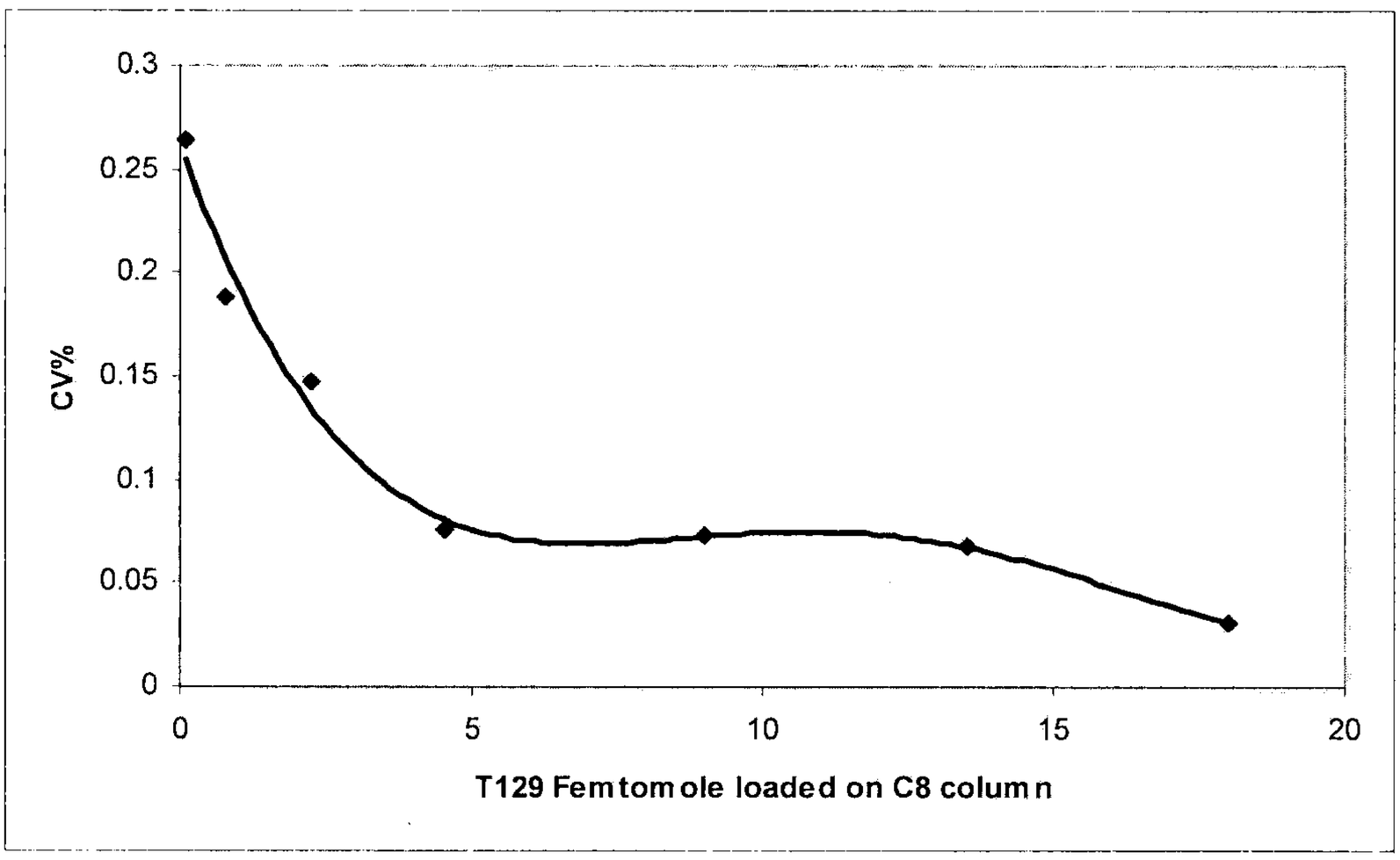

0.3
0.25
0.2
0.15
0.1
0.05
0
CV%
0
5
10
15
20
T129 Femtomole loaded on C8 column

Figure 6

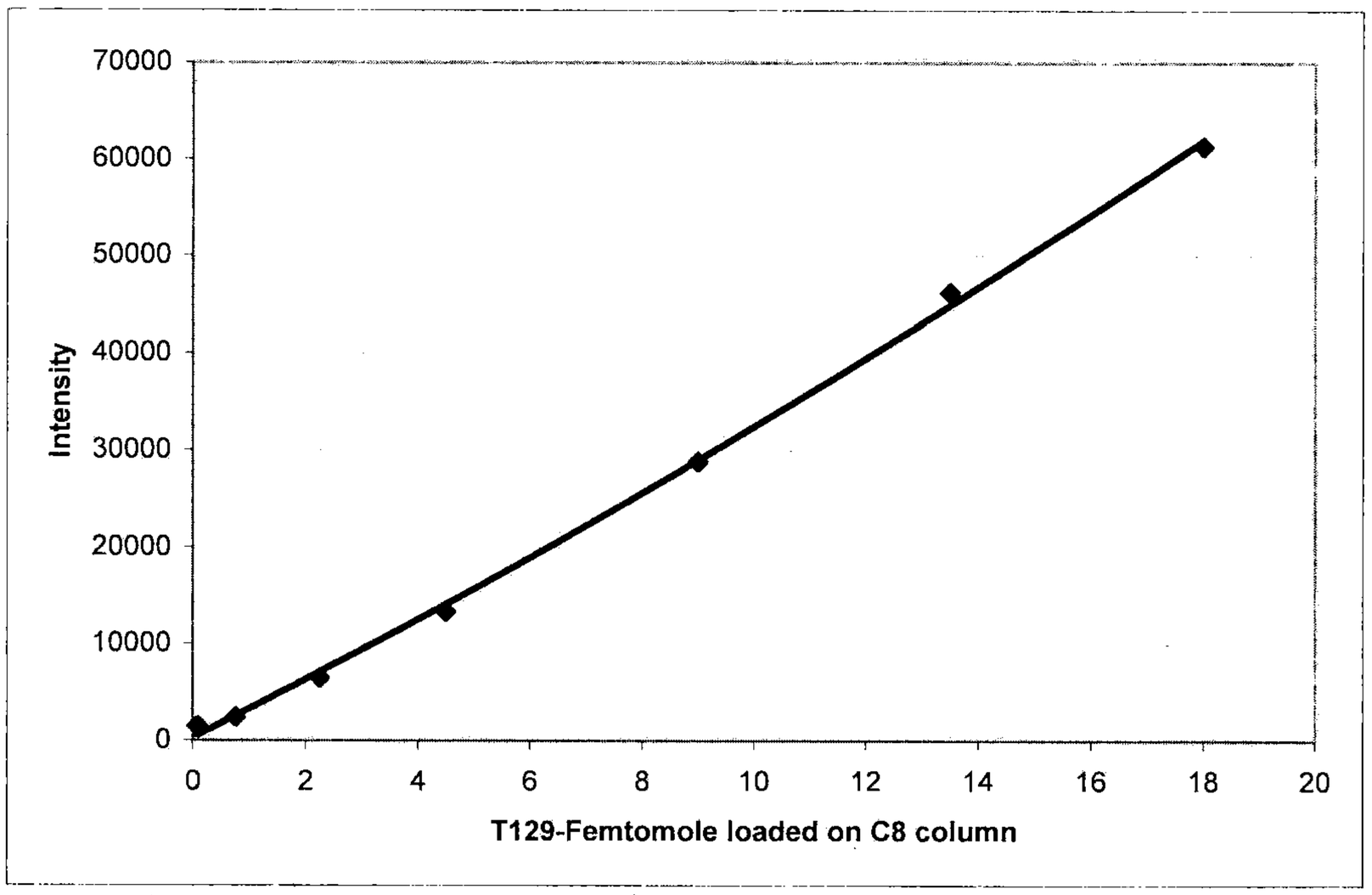

70000
60000
50000
40000
30000
20000
10000
0
Intensity
0
2
4
6
8
10
12
14
16
18
20
T129-Femtomole loaded on C8 column

Figure 7

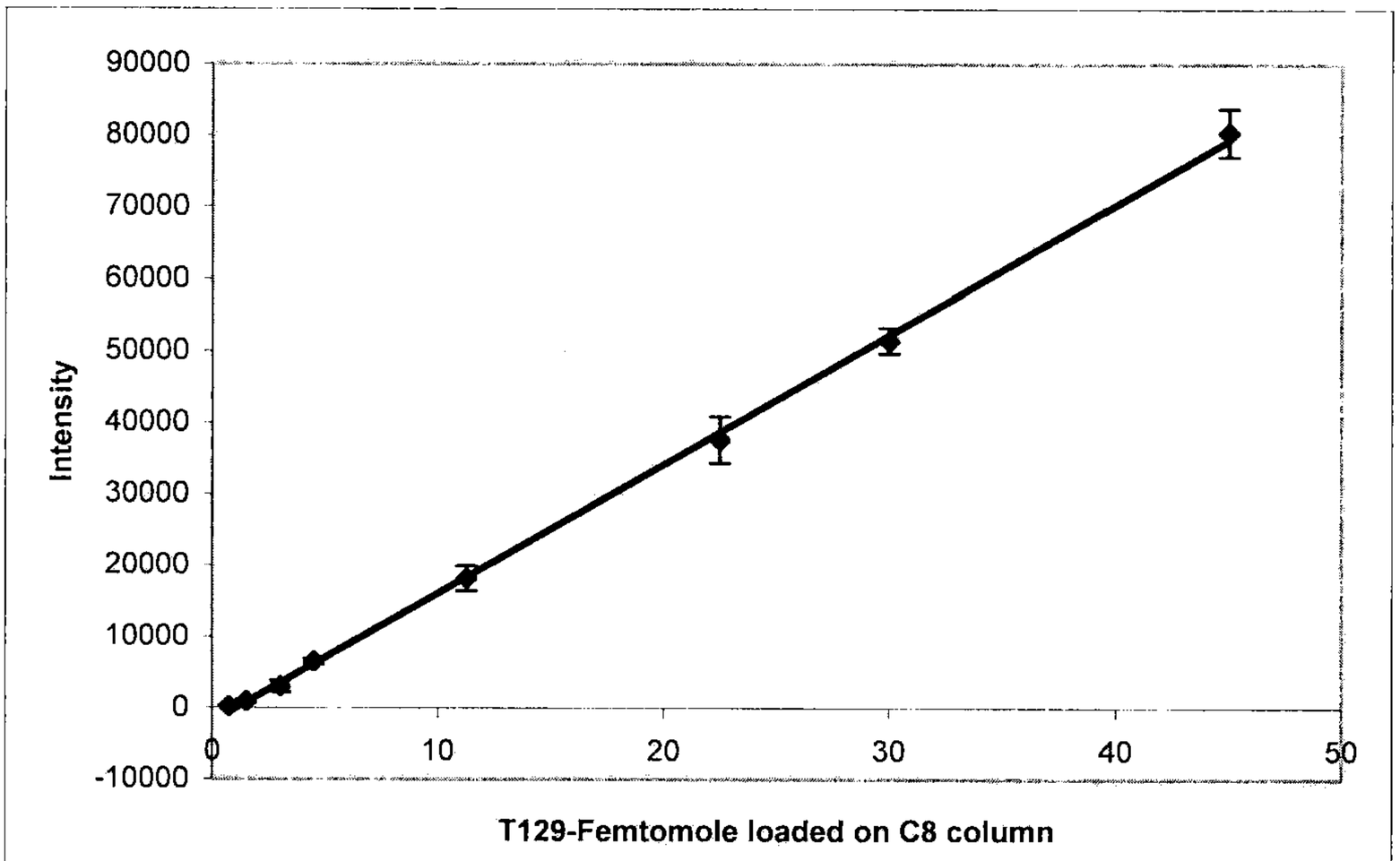

90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
Intensity
0
10
20
30
40
50
T129-Femtomole loaded on C8 column

Figure 8

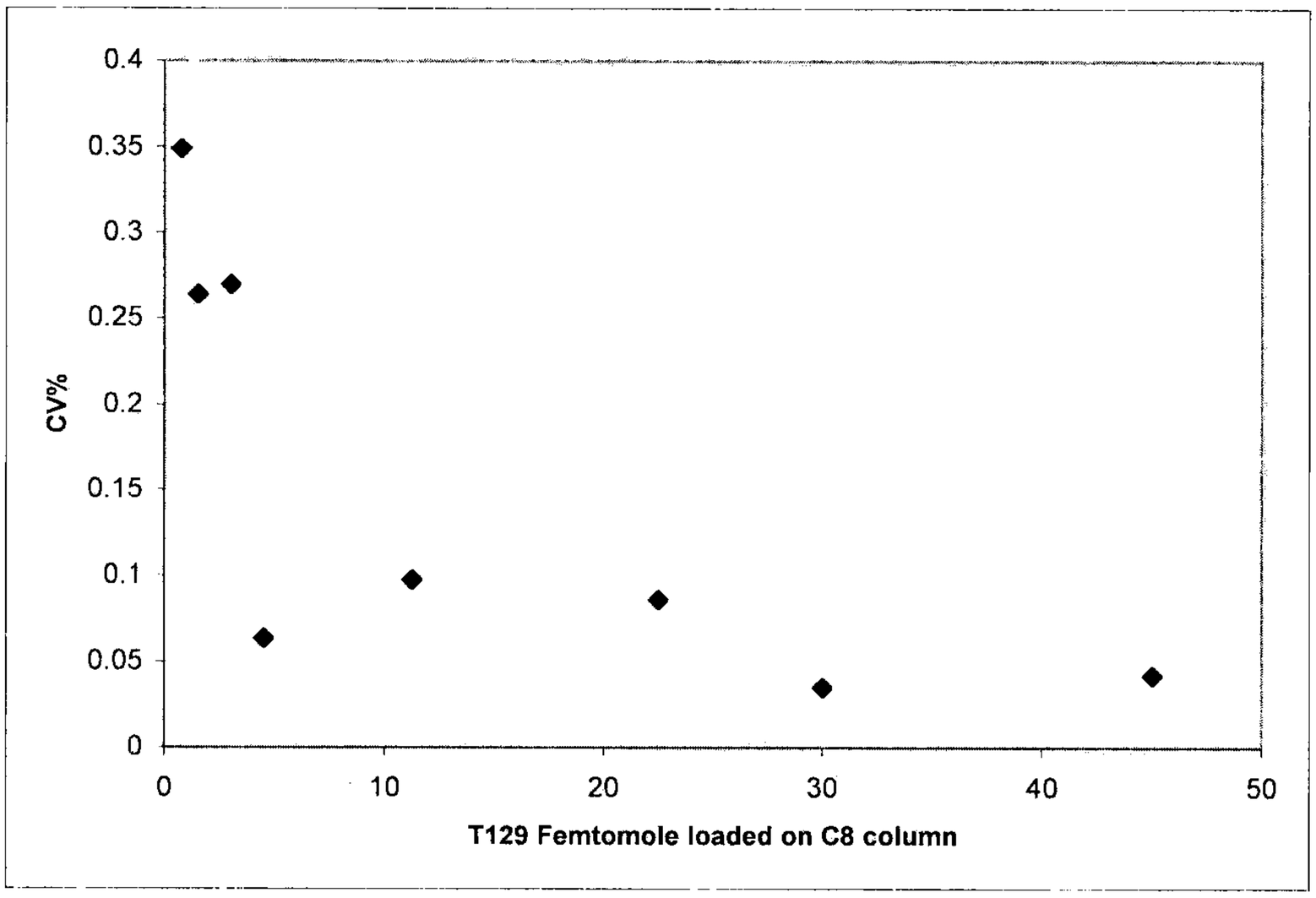
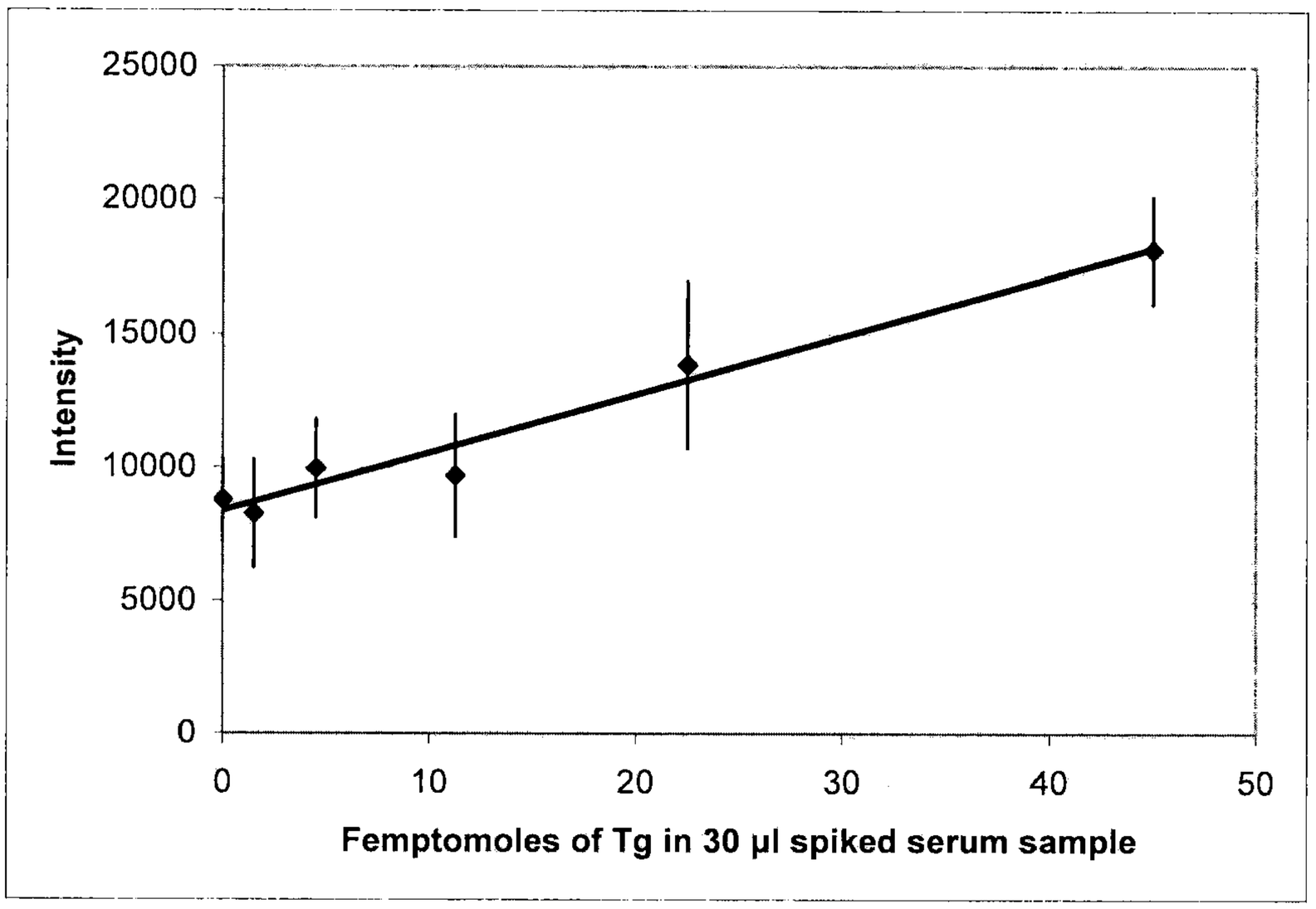

0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
CV%
0
10
20
30
40
50
T129 Femtomole loaded on C8 column

Figure 9


25000
20000
15000
10000
5000
0
Intensity
0
10
20
30
40
50
Femptomoles of Tg in 30 µl spiked serum sample

THYROGLOBULIN QUANTITATION BY MASS SPECTROMETRY

CROSS REFERENCE TO RELATED PATENT APPLICATIONS

This application claims priority under 35 U.S.C. §120 to U.S. application Ser. No. 12/001,076, filed Dec. 6, 2007, incorporated herein by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Sep. 15, 2011, is named 54769284.txt and is 62,398 bytes in size.

FIELD OF THE INVENTION

The invention relates to the quantitation of thyroglobulin. In a particular aspect, the invention relates to methods for quantitation of thyroglobulin by mass spectrometry.

BACKGROUND OF THE INVENTION

The following description of the background of the invention is provided simply as an aid in understanding the invention and is not admitted to describe or constitute prior art to the invention.

Thyroglobulin, or Tg, is a large dimeric secretary glycoprotein with a molecular weight of 660 kDa comprised of noncovalently bound homodimers.

Tg molecules exist in several forms. The three major Tg molecule sequences as found in the UniProt Knowledgebase (Swiss-Prot+TrEMBL) are P01266 (Human Thyroglobulin Precursor), P01266-2 (Isoform 2 of P01266), and Q59GF02 (Human Thyroglobulin Variant). (See FIGS. **1**, **2**, and **3**, respectively.)

P01266 is the major variant of P01266 with a length of 2768 AA; P01266-2 is an isoform of P01266 with a length of 2711 AA. P01266-2 varies from P01266 at amino acid positions 1510 to 1567 of Tg; and Q59GF0 is a thyroglobulin fragment with alength of 1574 AA. Q59GF0 contains amino acids from positions 1212 to 2768 of Tg.

Tg can only be produced in the thyroid gland and may be produced by either normal well differentiated benign thyroid cells or thyroid cancer cells. It is the precursor protein for thyroid hormone syntheses and serves as the matrix for thyroid iodine storage. Tg is used by the thyroid gland to produce the thyroid hormones thyroxine (T4) and triiodothyroine (T3). Tg levels in the blood can be used as a tumor marker for differentiated thyroid carcinoma (DTC). A high level of Tg in the blood is not by itself an indicator of thyroid cancer, but persistence of Tg in the blood following surgical removal of the thyroid gland indicates persistence of thyroid tissue. A course of treatment following detection of Tg in the blood following surgical removal of the thyroid gland may include administration of radioiodine to ablate all remaining normal thyroid. Continued persistence of Tg in the blood following ablation of all normal thyroid could indicate that some amount of tumor is still present.

Several methods for quantaition of Tg have been developed. For example Spencer, et al., Thyroid, 1999, 9(5):435-41 and Persoon, et al., Clinical Chem 2006, 52(4):686-691 disclose immunometric, radioimmunometric, and immunochemiluminometric methods for quantitation of Tg. These

methods are all subject to methodological problems such as differences in standardization, variability in interassay sensitivity and precision, hook effects, and interference attributable to Tg antibodies. The problem of interference attributable to Tg antibodies is particularly troubling for clinical application of monitoring Tg levels as a tumor marker because up to 20% of thyroid cancer patients have Tg autoantibodies.

SUMMARY OF THE INVENTION

The present invention provides methods for quantitation of Tg in a sample by mass spectrometry, including tandem mass spectrometry.

In one aspect, methods are provided for determining the amount of Tg in a test sample that include: (a) subjecting a Tg containing test sample to digestion resulting in creation of Tg peptides; (b) purifying one or more Tg peptides; (c) ionizing one or more Tg peptides; (d) detecting the amount of the Tg peptide ion(s) by mass spectrometry; and (e) relating the amount of detected Tg peptide ion(s) to the amount of Tg in the test sample. A preferred enzyme for preparing Tg peptides is trypsin. A suitable Tg peptide for the method is one that can be evaluated by mass spectrometry and can be sufficiently purified from related peptides that may be generated from proteins other than Tg. An example of one such peptide is peptide T129 (sequence VIFDANAPVAVR) (SEQ ID NO: 4) which contains amino acids from positions 1579 to 1590 of Tg, has a molecular weight of about 1,270 Da, and is present in all three isoforms of Tg. See FIG. **4**.

Formation of peptide T129 provides a unique trypsin generated peptide for thyroglobulin. Also, creation of peptide T129 from tryptic digestion of Tg should be unaffected by the presence or absence of the Tg antibodies. Thus, measurement of the increase in peptide T129 in a test sample offers a way of quantitating the amount of Tg originally in the test sample free from inference from Tg antibodies.

Any appropriate method may be used to determine the amount of Tg peptide resulting from digestion of Tg in a sample. In the event that a test sample may contain endogenous Tg peptide, steps may be taken to make certain that the endogenous peptide is not confused with peptide generated by digesting Tg in sample. One approach is to remove the endogenous Tg peptide from the sample before digesting Tg. This may done, for example, using a size separation technique. Another approach is to analyze a portion of a test sample according to the claimed methods but excluding the digestion step in order to establish a baseline level for the endogenous peptide in the test sample. In this approach, the once a baseline is determined, it can be subtracted from the post-digestion level of the peptide, the later representing both the endogenous peptide and that generated by digestion.

Because the methods may be applied to complex test samples (particularly body fluids or test samples derived from tissue), steps may be taken to purify Tg in the test sample prior to digestion. This may clone, for example, using a size separation technique.

In some embodiments, the methods include generating one or more Tg peptide ions in which at least one of the ions has a mass/charge ratio (m/z) corresponding to that of (singly or multiply charged) peptide T129 ions. In preferred related embodiments, the methods include generating one or more Tg peptide ions in which at least one has m/z of 1272.8±0.5, 636.4±0.5, or 424.3±0.5 (corresponding to singly, doubly, or triply charged peptide T129 ions). In related preferred embodiments, the methods may include generating one or more fragment ions of a Tg peptide ion in which at least one

has a m/z of 541.3±0.5, 612.3±0.5, 726.4±0.5, 797.4±0.5, 912.4±0.5, or 1059.5±0.5; preferably one or more of the fragment ions are selected from the group consisting of ions with a m/z of 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

In some embodiments, the purification in step (b) is accomplished with at least one size separation technique. Preferably, size separation techniques may be filtration, LC, or any combination thereof. In certain preferred embodiments, the test sample is a body fluid or tissue. In some embodiments, an additional step is included where a second quantity of the test sample is subjected to steps (b) through (e) in order to establish a baseline level of one or more endogenous Tg peptides. In these embodiments, this baseline level can be subtracted from the amount of Tg peptide ion(s) detected in the test sample to determine the amount of Tg peptide ion(s) that result from Tg in the original test sample. In other embodiments, the methods include an additional initial step of purifying Tg in the test sample prior to digestion. In these embodiments, the pre-digestion purification and/or the purification in step (b) may each be accomplished with at least one size separation technique. Preferably, at least one size separation technique used in both pre-digestion purification and step (b) is filtration; more preferably, this filtration is done with a molecular weight cut-off filter with molecular weigh cut off that allows for retention of Tg above the filter and allows Tg peptides to pass through with the filtrate. In related embodiments, the molecular weigh cut-off is about 2 kD to 300 kD; more preferably about 100 kD to 300 kD. In these embodiments, the two filtrations (pre-digestion and step (b)) may be conducted with the same filter.

In a second aspect, methods are provided for determining the amount of Tg in a test sample that include: (a) subjecting a Tg containing test sample to digestion resulting in creation of peptide T129; (b) purifying peptide T129; (c) ionizing peptide T129 to generate a precursor ion with a m/z of 636.4±0.5; (d) fragmenting the peptide T129 precursor ion to form one or more fragment ions in which at least one has a m/z of about 797.4±0.5, 912.4±0.5, or 1059.5±0.5; detecting the amount of peptide T129 precursor ions, one or more fragment ions, or both, by mass spectrometry; and (e) relating the amount of detected ion(s) to the amount of Tg in the test sample. In certain preferred embodiments, the test sample is a body fluid or tissue or tissue. In some embodiments, an additional step is included where a second quantity of the test sample is subjected to steps (b) through (e) in order to establish a baseline level of one or more endogenous peptide T129. In these embodiments, this baseline level can be subtracted from the amount of peptide T129 ion(s) detected in the test sample to determine the amount of peptide T129 ion(s) that result from Tg in the original test sample. In other embodiments, the methods include an additional initial step of purifying Tg in the test sample prior to digestion. In these embodiments, the pre-digestion purification and/or the purification in step (b) may each be accomplished with at least one size separation technique. Preferably, at least one size separation technique used in both pre-digestion purification and step (b) is filtration; more preferably, this filtration is done with a molecular weight cut-off filter with molecular weigh cut off that allows for retention of Tg above the filter and allows Tg peptides to pass through with the filtrate. In related embodiments, the molecular weigh cut-off is about 2 kD to 300 kD; more preferably about 100 kD to 300 kD. In these embodiments, the two filtrations (pre-digestion and step (b)) may be conducted with the same filter.

As used herein, the term "purification" or "purifying" does not refer to removing all materials from the sample other than the analyte(s) of interest. Instead, purification refers to a procedure that enriches the amount of one or more analytes of interest relative to one or more other components of the sample. Purification, as used herein, does not require the isolation of an analyte from all others. In preferred embodiments, a purification step or procedure can be used to remove one or more interfering substances, e.g., one or more substances that would interfere with the operation of the instruments used in the methods or substances that may interfere with the detection of an analyte ion by mass spectrometry.

As used herein, the term "about" in reference to quantitative measurements, not including the measurement of mass of an ion, refers to the indicated value plus or minus 10%.

As used herein, the term "substantially all" refers to any proportion greater than 50%, more preferably greater than 60%, more preferably greater than 70%, more preferably greater than 80%, and more preferably greater than 90%.

As used herein, the term "test sample" refers to any sample that may contain Tg. As used herein, the term "body fluid or tissue" means any fluid or tissue that can be isolated from the body of an individual. For example, "body fluid or tissue" may include blood, plasma, serum, bile, saliva, urine, tears, perspiration, and the like. If solid tissue is to be analyzed, it may be processed to release a liquid fraction that could contain any Tg present in the tissue. The liquid fraction can then be subject to the methods described herein.

As used herein, the term "digestion" means proteolytic cleavage of proteins into peptides. Digestion agents may include trypsin, Lyc-C, Arg-R, Asp-N and the like. Digestion is carried out by adding a digestion agent (i.e., an enzyme) to a sample and incubating for some period of time.

As used herein, "Tg" or "Tg molecule" means an intact Tg protein molecule.

As used herein, the term "Tg peptide" means any peptide of 100 amino acids or less that is a fragment of the native Tg. Tg peptides can be endogenous to a test sample or formed as a result of digestion of Tg. Peptide T129 is an example of a Tg peptide formed as a result of trypsin digestion of Tg.

As used herein, the term "size separation technique" means any technique (physical or chemical) that allows for the separation of at least one species from a test sample based on any one or more of molecular weight and shape. Examples of such techniques include, but are not limited to, filtration, chromatography, and certain aspects of mass spectrometry.

As used herein, the term "chromatography" refers to a process in which a chemical mixture carried by a liquid or gas is separated into components as a result of differential distribution of the chemical entities as they flow around, over, and/or through a stationary liquid or solid phase.

As used herein, the term "liquid chromatography" or "LC" means a process of selective retardation of one or more components of a fluid solution as the fluid uniformly percolates through a column of a finely divided substance, or through capillary passageways. The retardation results from the distribution of the components of the mixture between one or more stationary phases and the bulk fluid, (i.e., mobile phase), as this fluid moves relative to the stationary phase(s). "Liquid chromatography" includes reverse phase liquid chromatography (RPLC), high performance liquid chromatography (HPLC) and high turbulence liquid chromatography (HTLC).

As used herein, the term "high performance liquid chromatography" or "HPLC" refers to liquid chromatography in which the degree of separation is increased by forcing the mobile phase under pressure through a stationary phase, typically a densely packed column.

As used herein, the term "mass spectrometry" or "MS" refers to an analytical technique to identify compounds by their mass. MS refers to methods of filtering, detecting, and

measuring ions based on their m/z. MS technology generally includes (1) ionizing the compounds to form charged species (e.g., ions); and (2) detecting the molecular weight of the ions and calculating their m/z. The compounds may be ionized and detected by any suitable means. A "mass spectrometer" generally includes an ionizer and an ion detector. In general, one or more molecules of interest are ionized, and the ions are subsequently introduced into a mass spectrographic instrument where, due to a combination of magnetic and electric fields, the ions follow a path in space that is dependent upon mass ("m") and charge ("z"). See, e.g., U.S. Pat. Nos. 6,204,500, entitled "Mass Spectrometry From Surfaces;" 6,107,623, entitled "Methods and Apparatus for Tandem Mass Spectrometry;" 6,268,144, entitled "DNA Diagnostics Based On Mass Spectrometry;" 6,124,137, entitled "Surface-Enhanced Photolabile Attachment And Release For Desorption And Detection Of Analytes;" Wright et al., *Prostate Cancer and Prostatic Diseases* 2:264-76 (1999); and Merchant and Weinberger, *Electrophoresis* 21:1164-67 (2000).

As used herein, the term "operating in positive ion mode" refers to those mass spectrometry methods where positive ions are detected. Similarly, the term "operating in negative ion mode" refers to those mass spectrometry methods where negative ions are detected.

As used herein, the term "ionization" or "ionizing" refers to the process of generating an analyte ion having a net electrical charge equal to one or more electron units. Positive ions are those having a net positive charge of one or more electron units. Negative ions are those having a net negative charge of one or more electron units.

As used herein, the term "electron ionization" or "EI" refers to methods in which an analyte of interest in a gaseous or vapor phase interacts with a flow of electrons. Impact of the electrons with the analyte produces analyte ions, which may then be subjected to a mass spectrometry technique.

As used herein, the term "chemical ionization" or "CI" refers to methods in which a reagent gas (e.g. ammonia) is subjected to electron impact, and analyte ions are formed by the interaction of reagent gas ions and analyte molecules.

As used herein, the term "fast atom bombardment" or "FAB" refers to methods in which a beam of high energy atoms (often Xe or Ar) impacts a non-volatile sample, desorbing and ionizing molecules contained in the sample. Test samples are dissolved in a viscous liquid matrix such as glycerol, thioglycerol, m-nitrobenzyl alcohol, 18-crown-6 crown ether, 2-nitrophenyloctyl ether, sulfolane, diethanolamine, and triethanolamine. The choice of an appropriate matrix for a compound or sample is an empirical process.

As used herein, the term "matrix-assisted laser desorption ionization" or "MALDI" refers to methods in which a non-volatile sample is exposed to laser irradiation, which desorbs and ionizes analytes in the sample by various ionization pathways, including photo-ionization, protonation, deprotonation, and cluster decay. For MALDI, the sample is mixed with an energy-absorbing matrix, which facilitates desorption of analyte molecules.

As used herein, the term "surface enhanced laser desorption ionization" or "SELDI" refers to another method in which a non-volatile sample is exposed to laser irradiation, which desorbs and ionizes analytes in the sample by various ionization pathways, including photo-ionization, protonation, deprotonation, and cluster decay. For SELDI, the sample is typically bound to a surface that preferentially retains one or more analytes of interest. As in MALDI, this process may also employ an energy-absorbing material to facilitate ionization.

As used herein, the term "electrospray ionization" or "ESI," refers to methods in which a solution is passed along a short length of capillary tube, to the end of which is applied a high positive or negative electric potential. Solution reaching the end of the tube is vaporized (nebulized) into a jet or spray of very small droplets of solution in solvent vapor. This mist of droplets flows through an evaporation chamber, which is heated slightly to prevent condensation and to evaporate solvent. As the droplets get smaller the electrical surface charge density increases until such time that the natural repulsion between like charges causes ions as well as neutral molecules to be released.

As used herein, the term "atmospheric pressure chemical ionization" or "APCI," refers to mass spectrometry methods that are similar to ESI; however, APCI produces ions by ion-molecule reactions that occur within a plasma at atmospheric pressure. The plasma is maintained by an electric discharge between the spray capillary and a counter electrode. Then ions are typically extracted into the mass analyzer by use of a set of differentially pumped skimmer stages. A counterflow of dry and preheated N_2 gas may be used to improve removal of solvent. The gas-phase ionization in APCI can be more effective than ESI for analyzing less-polar species.

The term "Atmospheric Pressure Photoionization" or "APPI" as used herein refers to the form of mass spectrometry where the mechanism for the photoionization of molecule M is photon absorption and electron ejection to form the molecular M+. Because the photon energy typically is just above the ionization potential, the molecular ion is less susceptible to dissociation. In many cases it may be possible to analyze samples without the need for chromatography, thus saving significant time and expense. In the presence of water vapor or protic solvents, the molecular ion can extract H to form MH+. This tends to occur if M has a high proton affinity. This does not affect quantitation accuracy because the sum of M+ and MH+ is constant. Drug compounds in protic solvents are usually observed as MH+, whereas nonpolar compounds such as naphthalene or testosterone usually form M+. Robb, D. B., Covey, T. R. and Bruins, A. P. (2000): See, e.g., Robb et al., Atmospheric pressure photoionization: An ionization method for liquid chromatography-mass spectrometry. *Anal. Chem.* 72(15): 3653-3659.

As used herein, the term "inductively coupled plasma" or "ICP" refers to methods in which a sample is interacted with a partially ionized gas at a sufficiently high temperature to atomize and ionize most elements

As used, herein, the term "field desorption" refers to methods in which a non-volatile test sample is placed on an ionization surface, and an intense electric field is used to generate analyte ions.

As used herein, the term "desorption" refers to the removal of an analyte from a surface and/or the entry of an analyte into a gaseous phase.

As used herein, the term "limit of quantification" or "LOQ" refers to the point where measurements become quantitatively meaningful. The analyte response at this LOQ is identifiable, discrete and reproducible with a precision of 20% and an accuracy of 80% to 120%.

In certain preferred embodiments of the methods disclosed herein, mass spectrometry is performed in positive ion mode. In certain particularly preferred embodiments of the methods disclosed herein, mass spectrometry is performed using ESI as the method of creating ions from Tg peptides.

In preferred embodiments, the ions from Tg peptide ionization detectable in a mass spectrometer are selected from the group consisting of ions with a m/z of 636.4±0.5,

1059.5±0.5, 921.4±0.5, 797.4±0.5, 726.4±0.5, 612.3±0.5, and 541.3±0.5; the first ion listed (m/z of 636.4±0.5) being a precursor ion with a net charge of positive 2 electron units and the latter six ions listed being fragment ions of the precursor ion. In particularly preferred embodiments, the precursor ion has a net charge of positive 2 electron units and a m/z of about 636.4±0.5, and the fragment ions have a m/z of 1059.5±0.5, 921.4±0.5, or 797.4±0.5.

In some preferred embodiments, a separately detectable internal standard peptide (e.g., T129) is introduced in the test sample after trypsin digestion. In these embodiments, all or a portion of the peptide present in the test sample both from digestion of endogenous Tg and the addition of the internal standard are ionized to produce a plurality of ions detectable in a mass spectrometer, and one or more ions produced from the peptide ionization are detected in a mass spectrometer.

In other preferred embodiments, a separately detectable internal Tg standard is provided in the test sample prior to trypsin digestion. In these embodiments, all or a portion of both the endogenous Tg and the internal standard present in the test sample are digested by trypsin resulting in formation of Tg peptides. Tg peptides are ionized to produce a plurality of ions detectable in a mass spectrometer, and one or more ions produced from Tg peptide ionization are detected by mass spectrometry.

In preferred embodiments, the ions detectable in a mass spectrometer produced from the ionization of Tg peptides resulting from Tg digestion are selected from the group consisting of ions with a m/z of 636.4±0.5, 1059.5±0.5, 921.4±0.5, 797.4±0.5, 726.4±0.5, 612.3±0.5, and 541.3±0.5; the first ion listed (m/z of 636.4±0.5) being a precursor ion with a net charge of positive 2 electron units and the latter six ions listed being fragment ions of the precursor ion. In particularly preferred embodiments, the precursor ion has a net charge of positive 2 electron units and a m/z of 636.4±0.5, and the fragment ions have a m/z of 1059.5±0.5, 921.4±0.5, 797.4±0.5.

In preferred embodiments, the presence or amount of Tg peptide ions is related to the presence or amount of Tg in the original test sample by comparison to a reference Tg sample.

In one embodiment, the methods involve the combination of LC with mass spectrometry. In another preferred embodiment, the mass spectrometry is tandem mass spectrometry (MS/MS).

The summary of the invention described above is non-limiting and other features and advantages of the invention will be apparent from the following detailed description of the invention, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the amino acid sequence for P01266 (Human Thyroglobulin Precursor) (SEQ ID NO: 1).

FIG. **2** shows the amino acid sequence for P01266-2 (Isoform 2 of P01266) (SEQ ID NO: 2).

FIG. **3** shows the amino acid sequence for Q59GF0 (Thyroglobulin Variant-Fragment) (SEQ ID NO: 3).

FIG. **4** shows a comparison of the three sequences contained in FIGS. **1-3** demonstrating that they all contain amino acids corresponding to positions 1579 to 1590 of Tg. Sequence P01266 is on top (SEQ ID NO: 1); sequence P01266-2 is in the middle (SEQ ID NO: 2); and sequence Q59GF0 is at the bottom (SEQ ID NO: 3).

FIG. **5** shows the limit of quantitation verification for Tg peptide ion with m/z corresponding to peptide T129 by MS/MS. The equation describing the trend line for FIG. **5** is as follows: $y=1E\text{-}05x^4-0.0007x^3+0.0114x^2-0.0787x+0.2606$. $R^2=0.9833$ for this fit. Details are described in Example 1.

FIG. **6** shows the linearity of the quantitation of peptide T129 in serially diluted stock samples using an LC-MS/MS assay. The equation describing the trend line for FIG. **6** is as follows: $y=26.919x^2+2939.4x+310.78$. $R^2=0.9988$ for this fit. Details are described in Example 1.

FIG. **7** shows the limit of quantitation verification for peptide T129 in stripped serum by MS/MS. The equation describing the trend line for FIG. **7** is as follows: $y=1807.2x-1975$. $R^2=0.9993$ for this fit. Details are described in Example 2.

FIG. **8** shows the linearity of the quantitation of peptide T129 in peptide T129 spiked stripped serum using an LC-MS/MS assay. Details are described in Example 2.

FIG. **9** shows the linearity of the quantitation of Tg peptide ions with m/z corresponding to peptide T129 using an LC-MS/MS assay in stripped serum spiked with Tg prior to processing and concentration according to the methods described herein. The equation describing the trend line for FIG. **9** is as follows: $y=218.15x+8363.2$. $R^2=0.9681$ for this fit. Details are described in Example 3.

DETAILED DESCRIPTION OF THE INVENTION

Methods are described for quantitatively measuring Tg in a test sample. This quantitative measurement is achieved through the use of LC-MS/MS techniques. Prior to the use of LC-MS/MS, samples may be prepared by the following technique, or any portion thereof. A first purification of Tg in a test sample may be conducted through the use of a size separation technique such that substantially all Tg in the test sample is retained. Following the first purification step, enzymatic digestion of Tg may be carried out creating Tg peptides of interest. After digestion, another utilization of a size separation technique may be employed such that a selected Tg peptide generated in the enzymatic digestion of Tg is purified. This second size separation technique can be used to remove substantially all undigested, higher-molecular weight species. Properly executed, the sample preparation techniques ensure that selected Tg peptides quantitated by LC-MS/MS directly result from enzymatic digestion of Tg originally in the test sample; thus, the level of selected Tg peptides in the test sample at the start of LC-MS/MS is directly proportional to the amount of Tg originally present in the test sample.

Any suitable size separation technique may be utilized, but in the examples that follow, both the first and second size separation techniques are filtration through a molecular weight cut-off filter. It is also possible, as discussed in the Examples that follow, to select a molecular weight cut-off filter with an appropriate molecular weight cut-off such that the same filter can be used for both the first size separation and the second size separation.

LC, most preferably HPLC, is utilized, may be utilized either alone or in combination with other purification methods, to purify selected Tg peptides. This purification is combined with MS/MS, thereby providing an assay system for quantifying selected Tg peptides in a test sample. The quantity of the selected Tg peptides in the test sample is then used to determine the quantity of Tg in the original test sample. The Tg quantitation methods provided herein have enhanced specificity and are less subject to methodological problems (such as Tg antibody interference).

Suitable test samples may include any test sample that may contain the analyte of interest. In some preferred embodiments, a sample is a biological sample; that is, a sample

obtained from any biological source, such as an animal, a cell culture, an organ culture, and the like. In certain preferred embodiments, samples are obtained from a mammalian animal, such as a dog, cat, horse, etc. Particularly preferred mammalian animals are primates, most preferably humans. Particularly preferred samples include blood, plasma, serum, urine, saliva, tears, cerebrospinal fluid, or other body fluid or tissue samples. Such samples may be obtained, for example, from a patient; that is, a living person presenting oneself in a clinical setting for diagnosis, prognosis, or treatment of a disease or condition. The test sample is preferably obtained from a patient, for example, serum or plasma.

Sample Preparation for Mass Spectrometry

Samples may be processed or purified to obtain preparations that are suitable for analysis by mass spectrometry. Such purification will usually include chromatography, such as liquid chromatography, and may also often involve an additional purification procedure that is performed prior to chromatography. Various procedures may be used for this purpose depending on the type of sample or the type of chromatography. Examples include filtration, centrifugation, combinations thereof and the like. In certain preferred embodiments, Tg present in a test sample prior to enzymatic digestion.

Filtration is one preferred method of preparing a test sample, especially a biological test sample, such as serum or plasma, for chromatography. Such filtration is carried out by filtering a test sample through a molecular weight cut-off filter to separate species with molecular weights higher than the filter's cut-off (including Tg) from those with molecular weights lower than the filter's cut-off. The test sample remaining above the filter following complete (or near complete) filtration is substantially free of potentially interfering species with molecular weights lower than the filter's cut-off.

The pH of the test sample may then be adjusted to any point required by a digestion agent. In certain preferred embodiments, the digestion agent is trypsin and pH can be adjusted with a solution of ammonium acetate to have a pH suitable for this enzyme. In these preferred embodiments, the sample is then digested with trypsin to form Tg peptides (including peptide T129).

After trypsin digestion, the sample may be purified with a second filtration. This post-digestion filtration can be carried out similarly to the pre-digestion filtration described above (with the exception that the filtrate is retained), in order to separate Tg fragments from potentially interfering species with molecular weights higher than the filter's cut-off that may also be present in the sample. The filtrate from this post-digestion filtration can then be purified by liquid chromatography and subsequently subjected to mass spectrometry analysis.

Various methods have been described involving the use of HPLC for sample clean-up prior to mass spectrometry analysis. See, e.g., Taylor et al., *Therapeutic Drug Monitoring* 22:608-12 (2000) (manual precipitation of blood samples, followed by manual C18 solid phase extraction, injection into an HPLC for chromatography on a C18 analytical column, and MS/MS analysis); and Salm et al., *Clin. Therapeutics* 22 Supl. B:B71-B85 (2000) (manual precipitation of blood samples, followed by manual C18 solid phase extraction, injection into an HPLC for chromatography on a C18 analytical column, and MS/MS analysis). One of skill in the art may select HPLC instruments and columns that are suitable for use in the methods. The chromatographic column typically includes a medium (i.e., a packing material) to facilitate separation of chemical moieties (i.e., fractionation). The medium may include minute particles. The particles include a bonded surface that interacts with the various chemical moieties to facilitate separation of the chemical moieties. One suitable bonded surface is a hydrophobic bonded surface such as an alkyl bonded surface. Alkyl bonded surfaces may include C-4, C-8, or C-18 bonded alkyl groups, preferably C-8 bonded groups. The chromatographic column includes an inlet port for receiving a sample and an outlet port for discharging an effluent that includes the fractionated sample.

In certain embodiments, an analyte may be purified by applying a sample to a column under conditions where the analyte of interest is reversibly retained by the column packing material, while one or more other materials are not retained. In these embodiments, a first mobile phase condition can be employed where the analyte of interest is retained by the column and a second mobile phase condition can subsequently be employed to remove retained material from the column, once the non-retained materials are washed through. Alternatively, an analyte may be purified by applying a sample to a column under mobile phase conditions where the analyte of interest elutes at a differential rate in comparison to one or more other materials. Such procedures may enrich the amount of one or more analytes of interest relative to one or more other components of the sample.

In one embodiment, the sample to be analyzed is applied to the column at the inlet port, eluted with a solvent or solvent mixture, and discharged at the outlet port. Different solvent modes may be selected for eluting the analytes of interest. For example, liquid chromatography may be performed using a gradient mode, an isocratic mode, or a polytyptic (i.e. mixed) mode. In preferred embodiments, HPLC is performed on an analytical HPLC system with a C8 solid phase using 0.2% formic acid in HPLC Grade Ultra Pure Water and 0.2% formic acid in 100% methanol as the mobile phases.

Numerous column packings are available for chromatographic separation of samples and selection of an appropriate separation protocol is an empirical process that depends on the sample characteristics, analyte of interest, presence of interfering substances and their characteristics, etc. Commercially available HPLC columns include, but are not limited to, polar, ion exchange (both cation and anion), hydrophobic interaction, phenyl, C-2, C-8, C-18, and polar coating on porous polymer columns.

In one embodiment, the HPLC column has a C8 solid phase with a median particle size of 5 μm (nominal) and a median particle pore size of 100 Å. In a preferred embodiment the column dimensions are 1.0 mm ID×50 mm length (Phenomenex Corp. Luna 5μ C8(2) 100 Å New Column 50×1.0 mm, Phenomenex Cat. No. 00B-4249-A0 or equivalent).

During chromatography, the separation of materials is effected by variables such as choice of eluent (also known as a "mobile phase"), choice of gradient elution and the gradient conditions, temperature, etc.

Detection and Quantitation by Mass Spectrometry

In various embodiments, Tg peptides may be ionized by any method known to the skilled artisan. Mass spectrometry is performed using a mass spectrometer, which includes an ion source for ionizing the fractionated sample and creating charged molecules for further analysis. Ionization sources used in various MS techniques include, but are not limited to, electron ionization, chemical ionization, electrospray ionization (ESI), photon ionization, atmospheric pressure chemical ionization (APCI), photoionization, atmospheric pressure photoionization (APPI), fast atom bombardment (FAB)/liquid secondary ionization (LSIMS), matrix assisted laser desorption ionization (MALDI), field ionization, field desorption, thermospray/plasmaspray ionization, surface enhanced laser desorption ionization (SELDI), inductively coupled plasma (ICP) and particle beam ionization. The skilled artisan

will understand that the choice of ionization method may be determined based on the analyte to be measured, type of sample, the type of detector, the choice of positive versus negative mode, etc.

In preferred embodiments, Tg peptides are ionized by electrospray ionization (ESI) creating Tg peptide precursor ions. In related preferred embodiments, Tg peptide precursor ions are in a gaseous state and the inert collision gas is argon.

After the sample has been ionized, the positively charged ions thereby created may be analyzed to determine m/z. Suitable analyzers for determining m/z include quadrupole analyzers, ion trap analyzers, and time-of-flight analyzers. The ions may be detected using one of several detection modes. For example, only selected ions may be detected using a selective ion monitoring mode (SIM), or alternatively, multiple ions may be detected using a scanning mode, e.g., multiple reaction monitoring (MRM) or selected reaction monitoring (SRM). In preferred embodiments, ions are detected using SRM.

Preferably, m/z is determined using a quadrupole instrument. In a "quadrupole" or "quadrupole ion trap" instrument, ions in an oscillating radio frequency field experience a force proportional to the DC potential applied between electrodes, the amplitude of the RF signal, and m/z. The voltage and amplitude may be selected so that only ions having a particular m/z travel the length of the quadrupole, while all other ions are deflected. Thus, quadrupole instruments may act as both a "mass filter" and as a "mass detector" for the ions injected into the instrument.

One may enhance the resolution of the MS technique by employing "tandem mass spectrometry," or "MS/MS." In this technique, a precursor ion (also called a parent ion) generated from a molecule of interest can be filtered in an MS instrument, and the precursor ion subsequently fragmented to yield one or more fragment ions (also called daughter ions or product ions) that are then analyzed in a second MS procedure. By careful selection of precursor ions, only ions produced by certain analytes are passed to the fragmentation chamber, where collision with atoms of an inert gas produce the fragment ions. Because both the precursor and fragment ions are produced in a reproducible fashion under a given set of ionization/fragmentation conditions, the MS/MS technique may provide an extremely powerful analytical tool. For example, the combination of filtration/fragmentation may be used to eliminate interfering substances, and may be particularly useful in complex samples, such as biological samples.

Additionally, recent advances in technology, such as matrix-assisted laser desorption ionization coupled with time-of-flight analyzers ("MALDI-TOF") permit the analysis of analytes at femtomole levels in very short ion pulses. Mass spectrometers that combine time-of-flight analyzers with tandem MS are also well known to the artisan. Additionally, multiple mass spectrometry steps may be combined in methods known as "MS/MS". Various other combinations may be employed, such as MS/MS/TOF, MALDI/MS/MS/TOF, or SELDI/MS/MS/TOF mass spectrometry.

The mass spectrometer typically provides the user with an ion scan; that is, the relative abundance of each ion with a particular m/z over a given range (e.g., 400 to 1600 amu). The results of an analyte assay, that is, a mass spectrum, may be related to the amount of the analyte in the original sample by numerous methods known in the art. For example, given that sampling and analysis parameters are carefully controlled, the relative abundance of a given ion may be compared to a table that converts that relative abundance to an absolute amount of the original molecule. Alternatively, molecular standards may be run with the samples and a standard curve constructed based on ions generated from those standards. Using such a standard curve, the relative abundance of a given ion may be converted into an absolute amount of the original molecule. In certain preferred embodiments, an internal standard is used to generate a standard curve for calculating the quantity of Tg. Methods of generating and using such standard curves are well known in the art and one of ordinary skill is capable of selecting an appropriate internal standard. Numerous other methods for relating the amount of an ion to the amount of the original molecule will be well known to those of ordinary skill in the art.

One or more steps of the methods may be performed using automated machines. In certain embodiments, one or more purification steps are performed on-line, and more preferably all of the LC purification and mass spectrometry steps may be performed in an on-line fashion.

In certain embodiments, techniques such as MS/MS are used to isolate precursor ions for further fragmentation. In these embodiments, collision activation dissociation (CAD) may be used to generate the fragment ions for further detection. In CAD, precursor ions gain energy through collisions with an inert gas, and subsequently fragment by a process referred to as "unimolecular decomposition". Sufficient energy must be deposited in the precursor ion so that certain bonds within the ion can be broken due to increased vibrational energy. In alternative embodiments, electron transfer dissociation (ETD) may be used to generate the fragment ions. In ETD, radical anions are used to transfer electrons to multiply charged peptide or protein cations resulting in random cleavage along the peptide backbone.

In particularly preferred embodiments, Tg is detected and/or quantified using LC-MS/MS as follows. A Tg peptide enriched test sample prepared as described above is subjected to LC. The flow of liquid solvent from the chromatographic column enters the heated nebulizer interface of a LC-MS/MS analyzer and the solvent/analyte mixture is converted to vapor in the heated tubing of the interface. The analyte (e.g., Tg peptides), contained in the nebulized solvent, is ionized by the corona discharge needle of the interface, which applies a large voltage to the nebulized solvent/analyte mixture. The ions (i.e. Tg peptide precursor ions) pass through the orifice of the instrument and enter the first quadrupole. Quadrupoles 1 and 3 (Q1 and Q3) are mass filters, allowing selection of ions (i.e., "precursor" and "fragment" ions) based on their m/z. Quadrupole 2 (Q2) is the collision cell, where ions are fragmented. Q1 selects for ions with m/z of peptide T129 precursor ions (m/z of 636.4±0.5). Selected precursor ions are allowed to pass into the collision chamber (Q2), while ions with any other m/z collide with the sides of Q1 and are eliminated. Precursor ions entering Q2 may be fragmented with collision activated dissociation (CAD) through collisions with neutral argon gas molecules. Alternatively, if the precursor ions entering Q2 are multiply charged cations, they may be fragmented with electron transfer dissociation (ETD). The fragment ions generated are passed into Q3, where selected fragment ions are collected while other ions are eliminated.

Using standard methods well known in the art, one of ordinary skill is capable of identifying one or more fragment ions of a particular Tg peptide precursor ion that may be used for selection in Q3. A specific fragment ion is one that will not be formed in significant amounts by other molecules with similar molecular structures. In contrast, a non-specific fragment ion is one that is formed by molecules other than the desired analyte. Suitable specific fragment ions can be identified by testing various molecular standards to determine whether fragment ions formed by a selected Tg peptide are

also formed by other molecules with similar structures or features. Preferably, at least one fragment ion specific for Tg peptide ions with m/z corresponding to that of peptide T129 ions are identified. More preferably, one or more of these fragment ions have m/z of 797.4±0.5, 912.4±0.5 or 1059.5±0.5.

As ions collide with the detector they produce a pulse of electrons that are converted to a digital signal. The acquired data is relayed to a computer, which plots ion counts per unit time. The areas under the peaks corresponding to particular ions, or the amplitude of such peaks, are measured and the area or amplitude is correlated to the amount of the analyte of interest. In certain embodiments, the area under the curves, or amplitude of the peaks, for fragment ion(s) and/or precursor ions are measured to determine the amount of Tg peptides with m/z corresponding to peptide T129. As described above, the relative abundance of a given ion may be converted into an absolute amount of the original analyte using calibration standard curves based on peaks of one or more ions of an internal molecular standard. The absolute amount of an analyte detected by LC-MS/MS can then be converted into an absolute amount of Tg that was present in the original test sample.

The following examples serve to illustrate the invention. These examples are in no way intended to limit the scope of the methods.

EXAMPLES

Example 1

Demonstration of MS Quantitation of Peptide T129

Several samples with various known concentrations of peptide T129 were prepared by series dilution starting with a sample of known peptide T129 concentration. Peptide T129 LOQ and calibration curves were developed from LC-MS/MS analysis of these samples.

LC was performed with a Phenomenex analytical column (Phenomenex Corp. Luna 5µ. C8(2) 100 Å New Column 50×1.0 mm). A binary HPLC eluent composed of 0.2% formic acid in ultra pure water (HPLC grade) (mobile phase A) and 0.2% formic acid in 100% methanol (mobile phase B) was applied to the analytical column to separate selected Tg peptides from other species contained in the sample. The binary eluent was applied according to the following gradient profile: as a first step, an 80/20 mixture of mobile phase A/mobile phase B was applied for 120 seconds; as a second step, a 30/70 mixture of mobile phase A/mobile phase B was applied for 60 seconds; as a third step, the relative amount of mobile phase B in the mixture was ramped to a 5/95 mixture of mobile phase A/mobile phase B over a period of 120 seconds; as a fourth step, a 5/95 mixture of mobile phase A/mobile phase B was applied for 60 seconds; as a fifth and final step, an 80/20 mixture of mobile phase A/mobile phase B was applied for 240 seconds.

The separated sample was then subjected to MS/MS for quantitation of one or more Tg peptides with m/z corresponding to peptide T129.

MS/MS was performed using a Finnigan TSQ Quantum Ultra MS/MS system (Thermo Electron Corporation). The following software programs all from ThermoElectron were used in the Examples described herein: Tune Master V 1.2 or newer, Xcalibur V 2.0 SR1 or newer, TSQ Quantum 1.4 or newer, LCQuan V 2.0 or newer, and XReport 1.0 or newer. Liquid solvent/analyte exiting the analytical HPLC column flowed to the heated nebulizer interface of a Thermo Finnigan MS/MS analyzer. The solvent/analyte mixture was converted to vapor in the heated tubing of the interface. Analytes in the nebulized solvent were ionized by the corona discharge needle of the interface, which applied voltage to the nebulized solvent/analyte mixture.

Ions passed to the first quadrupole (Q1), which selected ions with a m/z of 636.4±0.5. Ions entering Quadrupole 2 (Q2) collided with argon gas to generate ion fragments, which were passed to quadrupole 3 (Q3) for further selection. Mass transitions used for quantitation of precursor ions with m/z corresponding to peptide T129 during validation on positive polarity are shown in Table 1.

TABLE 1

Mass transitions for precursor ions with m/z corresponding to peptide T129 (Positive Polarity)

Precursor Ion (m/z)	Fragment Ion (m/z)
636.4 ± 0.5	797.4 ± 0.5, 912.4 ± 0.5 & 1059.5 ± 0.5

To determine the limit of quantitation (LOQ) with a precision of 20% and an accuracy of 80% to 120%, seven different samples at varying concentrations were assayed and the reproducibility (CV) determined for each. The LOQ for one or more Tg peptides with m/z corresponding to peptide T129 was defined at about 67 amol/µl.

Data collected and used to develop the LOQ and Calibration curves in FIGS. **5** and **6** is shown in Table 2.

TABLE 2

Data collected and used to develop LOQ and Calibration curves for peptide T129 in spiked stripped serum samples

Peptide T129 Concentration (Attomoles/µl)	Femtomoles of peptide T129 in 30 µl sample	Average Ion Counts per Second	CV (%)
2.5	0.075	1471.6	0.264429
25	0.75	2435.6	0.188653
75	2.25	6455.4	0.147946
150	4.5	13322.4	0.075327
300	9	28805	0.073374
450	13.5	46199.6	0.067088
600	18	61302.2	0.030893

Example 2

Demonstration of Quantitation of Peptide T129 in Peptide T129 Spiked Processed, Concentrated and Digested Stripped Serum

A 500 µl sample of stripped serum (e.g., the test sample in this Example) was added atop the filter element of a commercially available 300 kDa molecular weight cut-off filter cartridge (Pall Corp. Nanosep 300 kDa, Pall Corp. Cat. No. OD300C33).

The test sample was completely filtered upon centrifugation of the cartridge at 13 kg for 6 minutes. The filtrate was removed and discarded. 500 µl of HPLC grade water was then added to the top of the filter and the cartridge was again centrifuged at 13 kg for 6 minutes. The filtrate was again removed and discarded. Next, 200 µl of 20 mM ammonium acetate was added to the top of the filter. The cartridge was again centrifuged at 13 kg for 3 minutes. The filtrate was again removed and discarded and 100 µl of 20 mM ammonium acetate was added to the top of the filter.

Then, 15 μg of trypsin (Promega Trypsin Gold, Mass Spec Grade, Promega Corp. Cat. No. V5280 or equivalent) was added to the test sample remaining on top of the filter. The resulting mixture was incubated without removal from the filter cartridge at 37 C for up to 17 hours.

After incubation, the filter cartridge was centrifuged at 13 kg for 6 minutes, and the filtrate retained. The filter cartridge was then washed by adding 50 μl of 20 mM ammonium acetate to the top of the filter and centrifuged at 13 kg for 6 minutes. Test samples for analysis by LC-MS/MS were created by pooling the two retained post-digestion filtrates.

The starting volume of stripped serum samples subjected to the above processing and concentration was about 500 μl. The final volume of each pooled post-digestion filtrate was about 130 μl. Thus the above process concentrates samples by a factor of 3.83.

Peptide T129 was then added to the pooled post-digestion filtrates in varying concentrations. 30 μl samples were then analyzed for quantitation of peptide T129 by LC-MS/MS according to the procedure described in Example 1 with the exception that the mass transitions shown in Table 3 were used. The fragment ion with a m/z of 797.4±0.5 was not used due to increased background generated by the processed, concentrated stripped serum.

TABLE 3

Mass transitions for precursor ions with m/z corresponding to peptide T129 from peptide T129 spiked stripped serum samples (Positive Polarity)

Precursor Ion (m/z)	Fragment Ion (m/z)
636.4 ± 0.5	912.4 ± 0.5 & 1059.5 ± 0.5

Data collected and used to develop the LOQ and Calibration curves found in FIGS. **7** and **8** is shown in Table 4.

TABLE 4

Data collected and used to develop LOQ and Calibration curves for peptide T129

Femtomoles of Tg in spiked serum sample	Average Ion Counts per Second	CV (%)
0.75	203	0.348839
1.5	957.25	0.263782
3	2984.75	0.269659
4.5	6504.75	0.063318
11.25	18210.5	0.097296
22.5	37620	0.085823
30	51451	0.035083

Example 3

Demonstration of Quantitation of Peptide T129 in Stripped Serum Containing Various Concentrations of Added Tg

Several 500 μl samples of stripped serum containing various concentrations of added Tg were prepared according to the procedure detailed in Example 2. LC-MS/MS of the resulting test samples was carried out following the steps detailed in Example 1.

Data collected and used to develop the calibration curve found in FIG. **9** are found in Table 6.

TABLE 6

Data collected and used to develop the calibration curve for peptide T129 MS/MS in Tg spiked stripped serum (processed and condensed as described in Example 3).

Femtomoles of Tg in spiked serum sample	Average Ion Counts per Second	CV (%)
0	8784.667	0.176987
1.5	8259.5	0.246833
4.5	9953.25	0.186588
11.25	9696.25	0.23816
22.5	13848.25	0.225496
45	18125.5	0.110826

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The methods illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including," containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the invention embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the methods. This includes the generic description of the methods with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the methods are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 4

<210> SEQ ID NO 1
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<213> ORGANISM: Homo sapiens

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Met Ala Leu Val Leu Glu Ile Phe Thr Leu Leu Ala Ser Ile Cys Trp
1               5                  10                  15

Val Ser Ala Asn Ile Phe Glu Tyr Gln Val Asp Ala Gln Pro Leu Arg
            20                  25                  30

Pro Cys Glu Leu Gln Arg Glu Thr Ala Phe Leu Lys Gln Ala Asp Tyr
        35                  40                  45

Val Pro Gln Cys Ala Glu Asp Gly Ser Phe Gln Thr Val Gln Cys Gln
    50                  55                  60

Asn Asp Gly Arg Ser Cys Trp Cys Val Gly Ala Asn Gly Ser Glu Val
65                  70                  75                  80

Leu Gly Ser Arg Gln Pro Gly Arg Pro Val Ala Cys Leu Ser Phe Cys
                85                  90                  95

Gln Leu Gln Lys Gln Gln Ile Leu Leu Ser Gly Tyr Ile Asn Ser Thr
            100                 105                 110

Asp Thr Ser Tyr Leu Pro Gln Cys Gln Asp Ser Gly Asp Tyr Ala Pro
        115                 120                 125

Val Gln Cys Asp Val Gln Gln Val Gln Cys Trp Cys Val Asp Ala Glu
    130                 135                 140

Gly Met Glu Val Tyr Gly Thr Arg Gln Leu Gly Arg Pro Lys Arg Cys
145                 150                 155                 160

Pro Arg Ser Cys Glu Ile Arg Asn Arg Arg Leu Leu His Gly Val Gly
                165                 170                 175

Asp Lys Ser Pro Pro Gln Cys Ser Ala Glu Gly Glu Phe Met Pro Val
            180                 185                 190

Gln Cys Lys Phe Val Asn Thr Thr Asp Met Met Ile Phe Asp Leu Val
        195                 200                 205

His Ser Tyr Asn Arg Phe Pro Asp Ala Phe Val Thr Phe Ser Ser Phe
    210                 215                 220

Gln Arg Arg Phe Pro Glu Val Ser Gly Tyr Cys His Cys Ala Asp Ser
225                 230                 235                 240

Gln Gly Arg Glu Leu Ala Glu Thr Gly Leu Glu Leu Leu Leu Asp Glu
                245                 250                 255

Ile Tyr Asp Thr Ile Phe Ala Gly Leu Asp Leu Pro Ser Thr Phe Thr
            260                 265                 270

Glu Thr Thr Leu Tyr Arg Ile Leu Gln Arg Arg Phe Leu Ala Val Gln
        275                 280                 285

Ser Val Ile Ser Gly Arg Phe Arg Cys Pro Thr Lys Cys Glu Val Glu
    290                 295                 300

Arg Phe Thr Ala Thr Ser Phe Gly His Pro Tyr Val Pro Ser Cys Arg
305                 310                 315                 320

Arg Asn Gly Asp Tyr Gln Ala Val Gln Cys Gln Thr Glu Gly Pro Cys
                325                 330                 335

Trp Cys Val Asp Ala Gln Gly Lys Glu Met His Gly Thr Arg Gln Gln
            340                 345                 350

Gly Glu Pro Pro Ser Cys Ala Glu Gly Gln Ser Cys Ala Ser Glu Arg
        355                 360                 365
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Gln Gln Ala Leu Ser Arg Leu Tyr Phe Gly Thr Ser Gly Tyr Phe Ser
    370                 375                 380

Gln His Asp Leu Phe Ser Ser Pro Glu Lys Arg Trp Ala Ser Pro Arg
385                 390                 395                 400

Val Ala Arg Phe Ala Thr Ser Cys Pro Pro Thr Ile Lys Glu Leu Phe
                405                 410                 415

Val Asp Ser Gly Leu Leu Arg Pro Met Val Glu Gly Gln Ser Gln Gln
            420                 425                 430

Phe Ser Val Ser Glu Asn Leu Leu Lys Glu Ala Ile Arg Ala Ile Phe
        435                 440                 445

Pro Ser Arg Gly Leu Ala Arg Leu Ala Leu Gln Phe Thr Thr Asn Pro
    450                 455                 460

Lys Arg Leu Gln Gln Asn Leu Phe Gly Gly Lys Phe Leu Val Asn Val
465                 470                 475                 480

Gly Gln Phe Asn Leu Ser Gly Ala Leu Gly Thr Arg Gly Thr Phe Asn
                485                 490                 495

Phe Ser Gln Phe Phe Gln Gln Leu Gly Leu Ala Ser Phe Leu Asn Gly
            500                 505                 510

Gly Arg Gln Glu Asp Leu Ala Lys Pro Leu Ser Val Gly Leu Asp Ser
        515                 520                 525

Asn Ser Ser Thr Gly Thr Pro Glu Ala Ala Lys Lys Asp Gly Thr Met
    530                 535                 540

Asn Lys Pro Thr Val Gly Ser Phe Gly Phe Glu Ile Asn Leu Gln Glu
545                 550                 555                 560

Asn Gln Asn Ala Leu Lys Phe Leu Ala Ser Leu Leu Glu Leu Pro Glu
                565                 570                 575

Phe Leu Leu Phe Leu Gln His Ala Ile Ser Val Pro Glu Asp Val Ala
            580                 585                 590

Arg Asp Leu Gly Asp Val Met Glu Thr Val Leu Ser Ser Gln Thr Cys
        595                 600                 605

Glu Gln Thr Pro Glu Arg Leu Phe Val Pro Ser Cys Thr Thr Glu Gly
    610                 615                 620

Ser Tyr Glu Asp Val Gln Cys Phe Ser Gly Glu Cys Trp Cys Val Asn
625                 630                 635                 640

Ser Trp Gly Lys Glu Leu Pro Gly Ser Arg Val Arg Gly Gly Gln Pro
                645                 650                 655

Arg Cys Pro Thr Asp Cys Glu Lys Gln Arg Ala Arg Met Gln Ser Leu
            660                 665                 670

Met Gly Ser Gln Pro Ala Gly Ser Thr Leu Phe Val Pro Ala Cys Thr
        675                 680                 685

Ser Glu Gly His Phe Leu Pro Val Gln Cys Phe Asn Ser Glu Cys Tyr
    690                 695                 700

Cys Val Asp Ala Glu Gly Gln Ala Ile Pro Gly Thr Arg Ser Ala Ile
705                 710                 715                 720

Gly Lys Pro Lys Lys Cys Pro Thr Pro Cys Gln Leu Gln Ser Glu Gln
                725                 730                 735

Ala Phe Leu Arg Thr Val Gln Ala Leu Leu Ser Asn Ser Ser Met Leu
            740                 745                 750

Pro Thr Leu Ser Asp Thr Tyr Ile Pro Gln Cys Ser Thr Asp Gly Gln
        755                 760                 765

Trp Arg Gln Val Gln Cys Asn Gly Pro Pro Glu Gln Val Phe Glu Leu
    770                 775                 780

Tyr Gln Arg Trp Glu Ala Gln Asn Lys Gly Gln Asp Leu Thr Pro Ala
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785                 790                 795                 800

Lys Leu Leu Val Lys Ile Met Ser Tyr Arg Glu Ala Ala Ser Gly Asn
                805                 810                 815

Phe Ser Leu Phe Ile Gln Ser Leu Tyr Glu Ala Gly Gln Gln Asp Val
            820                 825                 830

Phe Pro Val Leu Ser Gln Tyr Pro Ser Leu Gln Asp Val Pro Leu Ala
        835                 840                 845

Ala Leu Glu Gly Lys Arg Pro Gln Pro Arg Glu Asn Ile Leu Leu Glu
    850                 855                 860

Pro Tyr Leu Phe Trp Gln Ile Leu Asn Gly Gln Leu Ser Gln Tyr Pro
865                 870                 875                 880

Gly Ser Tyr Ser Asp Phe Ser Thr Pro Leu Ala His Phe Asp Leu Arg
                885                 890                 895

Asn Cys Trp Cys Val Asp Glu Ala Gly Gln Glu Leu Glu Gly Met Arg
            900                 905                 910

Ser Glu Pro Ser Lys Leu Pro Thr Cys Pro Gly Ser Cys Glu Glu Ala
        915                 920                 925

Lys Leu Arg Val Leu Gln Phe Ile Arg Glu Thr Glu Glu Ile Val Ser
    930                 935                 940

Ala Ser Asn Ser Ser Arg Phe Pro Leu Gly Glu Ser Phe Leu Val Ala
945                 950                 955                 960

Lys Gly Ile Arg Leu Arg Asn Glu Asp Leu Gly Leu Pro Pro Leu Phe
                965                 970                 975

Pro Pro Arg Glu Ala Phe Ala Glu Gln Phe Leu Arg Gly Ser Asp Tyr
            980                 985                 990

Ala Ile Arg Leu Ala Ala Gln Ser  Thr Leu Ser Phe Tyr  Gln Arg Arg
        995                 1000                1005

Arg Phe  Ser Pro Asp Asp Ser  Ala Gly Ala Ser Ala  Leu Leu Arg
    1010                1015                1020

Ser Gly  Pro Tyr Met Pro Gln  Cys Asp Ala Phe Gly  Ser Trp Glu
    1025                1030                1035

Pro Val  Gln Cys His Ala Gly  Thr Gly His Cys Trp  Cys Val Asp
    1040                1045                1050

Glu Lys  Gly Gly Phe Ile Pro  Gly Ser Leu Thr Ala  Arg Ser Leu
    1055                1060                1065

Gln Ile  Pro Gln Cys Pro Thr  Thr Cys Glu Lys Ser  Arg Thr Ser
    1070                1075                1080

Gly Leu  Leu Ser Ser Trp Lys  Gln Ala Arg Ser Gln  Glu Asn Pro
    1085                1090                1095

Ser Pro  Lys Asp Leu Phe Val  Pro Ala Cys Leu Glu  Thr Gly Glu
    1100                1105                1110

Tyr Ala  Arg Leu Gln Ala Ser  Gly Ala Gly Thr Trp  Cys Val Asp
    1115                1120                1125

Pro Ala  Ser Gly Glu Glu Leu  Arg Pro Gly Ser Ser  Ser Ser Ala
    1130                1135                1140

Gln Cys  Pro Ser Leu Cys Asn  Val Leu Lys Ser Gly  Val Leu Ser
    1145                1150                1155

Arg Arg  Val Ser Pro Gly Tyr  Val Pro Ala Cys Arg  Ala Glu Asp
    1160                1165                1170

Gly Gly  Phe Ser Pro Val Gln  Cys Asp Gln Ala Gln  Gly Ser Cys
    1175                1180                1185

Trp Cys  Val Met Asp Ser Gly  Glu Glu Val Pro Gly  Thr Arg Val
    1190                1195                1200
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Thr Gly  Gly Gln Pro Ala Cys  Glu Ser Pro Arg Cys  Pro Leu Pro
    1205                 1210                 1215

Phe Asn  Ala Ser Glu Val Val  Gly Gly Thr Ile Leu  Cys Glu Thr
    1220                 1225                 1230

Ile Ser  Gly Pro Thr Gly Ser  Ala Met Gln Gln Cys  Gln Leu Leu
    1235                 1240                 1245

Cys Arg  Gln Gly Ser Trp Ser  Val Phe Pro Pro Gly  Pro Leu Ile
    1250                 1255                 1260

Cys Ser  Leu Glu Ser Gly Arg  Trp Glu Ser Gln Leu  Pro Gln Pro
    1265                 1270                 1275

Arg Ala  Cys Gln Arg Pro Gln  Leu Trp Gln Thr Ile  Gln Thr Gln
    1280                 1285                 1290

Gly His  Phe Gln Leu Gln Leu  Pro Pro Gly Lys Met  Cys Ser Ala
    1295                 1300                 1305

Asp Tyr  Ala Asp Leu Leu Gln  Thr Phe Gln Val Phe  Ile Leu Asp
    1310                 1315                 1320

Glu Leu  Thr Ala Arg Gly Phe  Cys Gln Ile Gln Val  Lys Thr Phe
    1325                 1330                 1335

Gly Thr  Leu Val Ser Ile Pro  Val Cys Asn Asn Ser  Ser Val Gln
    1340                 1345                 1350

Val Gly  Cys Leu Thr Arg Glu  Arg Leu Gly Val Asn  Val Thr Trp
    1355                 1360                 1365

Lys Ser  Arg Leu Glu Asp Ile  Pro Val Ala Ser Leu  Pro Asp Leu
    1370                 1375                 1380

His Asp  Ile Glu Arg Ala Leu  Val Gly Lys Asp Leu  Leu Gly Arg
    1385                 1390                 1395

Phe Thr  Asp Leu Ile Gln Ser  Gly Ser Phe Gln Leu  His Leu Asp
    1400                 1405                 1410

Ser Lys  Thr Phe Pro Ala Glu  Thr Ile Arg Phe Leu  Gln Gly Asp
    1415                 1420                 1425

His Phe  Gly Thr Ser Pro Arg  Thr Trp Phe Gly Cys  Ser Glu Gly
    1430                 1435                 1440

Phe Tyr  Gln Val Leu Thr Ser  Glu Ala Ser Gln Asp  Gly Leu Gly
    1445                 1450                 1455

Cys Val  Lys Cys Pro Glu Gly  Ser Tyr Ser Gln Asp  Glu Glu Cys
    1460                 1465                 1470

Ile Pro  Cys Pro Val Gly Phe  Tyr Gln Glu Gln Ala  Gly Ser Leu
    1475                 1480                 1485

Ala Cys  Val Pro Cys Pro Val  Gly Arg Thr Thr Ile  Ser Ala Gly
    1490                 1495                 1500

Ala Phe  Ser Gln Thr His Cys  Val Thr Asp Cys Gln  Arg Asn Glu
    1505                 1510                 1515

Ala Gly  Leu Gln Cys Asp Gln  Asn Gly Gln Tyr Arg  Ala Ser Gln
    1520                 1525                 1530

Lys Asp  Arg Gly Ser Gly Lys  Ala Phe Cys Val Asp  Gly Glu Gly
    1535                 1540                 1545

Arg Arg  Leu Pro Trp Trp Glu  Thr Glu Ala Pro Leu  Glu Asp Ser
    1550                 1555                 1560

Gln Cys  Leu Met Met Gln Lys  Phe Glu Lys Val Pro  Glu Ser Lys
    1565                 1570                 1575

Val Ile  Phe Asp Ala Asn Ala  Pro Val Ala Val Arg  Ser Lys Val
    1580                 1585                 1590

Pro Asp  Ser Glu Phe Pro Val  Met Gln Cys Leu Thr  Asp Cys Thr
    1595                 1600                 1605
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Glu Asp  Glu Ala Cys Ser Phe  Phe Thr Val Ser Thr  Thr Glu Pro
    1610                 1615                 1620

Glu Ile  Ser Cys Asp Phe Tyr  Ala Trp Thr Ser Asp  Asn Val Ala
    1625                 1630                 1635

Cys Met  Thr Ser Asp Gln Lys  Arg Asp Ala Leu Gly  Asn Ser Lys
    1640                 1645                 1650

Ala Thr  Ser Phe Gly Ser Leu  Arg Cys Gln Val Lys  Val Arg Ser
    1655                 1660                 1665

His Gly  Gln Asp Ser Pro Ala  Val Tyr Leu Lys Lys  Gly Gln Gly
    1670                 1675                 1680

Ser Thr  Thr Thr Leu Gln Lys  Arg Phe Glu Pro Thr  Gly Phe Gln
    1685                 1690                 1695

Asn Met  Leu Ser Gly Leu Tyr  Asn Pro Ile Val Phe  Ser Ala Ser
    1700                 1705                 1710

Gly Ala  Asn Leu Thr Asp Ala  His Leu Phe Cys Leu  Leu Ala Cys
    1715                 1720                 1725

Asp Arg  Asp Leu Cys Cys Asp  Gly Phe Val Leu Thr  Gln Val Gln
    1730                 1735                 1740

Gly Gly  Ala Ile Ile Cys Gly  Leu Leu Ser Ser Pro  Ser Val Leu
    1745                 1750                 1755

Leu Cys  Asn Val Lys Asp Trp  Met Asp Pro Ser Glu  Ala Trp Ala
    1760                 1765                 1770

Asn Ala  Thr Cys Pro Gly Val  Thr Tyr Asp Gln Glu  Ser His Gln
    1775                 1780                 1785

Val Ile  Leu Arg Leu Gly Asp  Gln Glu Phe Ile Lys  Ser Leu Thr
    1790                 1795                 1800

Pro Leu  Glu Gly Thr Gln Asp  Thr Phe Thr Asn Phe  Gln Gln Val
    1805                 1810                 1815

Tyr Leu  Trp Lys Asp Ser Asp  Met Gly Ser Arg Pro  Glu Ser Met
    1820                 1825                 1830

Gly Cys  Arg Lys Asp Thr Val  Pro Arg Pro Ala Ser  Pro Thr Glu
    1835                 1840                 1845

Ala Gly  Leu Thr Thr Glu Leu  Phe Ser Pro Val Asp  Leu Asn Gln
    1850                 1855                 1860

Val Ile  Val Asn Gly Asn Gln  Ser Leu Ser Ser Gln  Lys His Trp
    1865                 1870                 1875

Leu Phe  Lys His Leu Phe Ser  Ala Gln Gln Ala Asn  Leu Trp Cys
    1880                 1885                 1890

Leu Ser  Arg Cys Val Gln Glu  His Ser Phe Cys Gln  Leu Ala Glu
    1895                 1900                 1905

Ile Thr  Glu Ser Ala Ser Leu  Tyr Phe Thr Cys Thr  Leu Tyr Pro
    1910                 1915                 1920

Glu Ala  Gln Val Cys Asp Asp  Ile Met Glu Ser Asn  Ala Gln Gly
    1925                 1930                 1935

Cys Arg  Leu Ile Leu Pro Gln  Met Pro Lys Ala Leu  Phe Arg Lys
    1940                 1945                 1950

Lys Val  Ile Leu Glu Asp Lys  Val Lys Asn Phe Tyr  Thr Arg Leu
    1955                 1960                 1965

Pro Phe  Gln Lys Leu Met Gly  Ile Ser Ile Arg Asn  Lys Val Pro
    1970                 1975                 1980

Met Ser  Glu Lys Ser Ile Ser  Asn Gly Phe Phe Glu  Cys Glu Arg
    1985                 1990                 1995

Arg Cys  Asp Ala Asp Pro Cys  Cys Thr Gly Phe Gly  Phe Leu Asn
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    2000                2005                2010

Val Ser  Gln Leu Lys Gly Gly  Glu Val Thr Cys Leu  Thr Leu Asn
    2015                2020                2025

Ser Leu  Gly Ile Gln Met Cys  Ser Glu Glu Asn Gly  Gly Ala Trp
    2030                2035                2040

Arg Ile  Leu Asp Cys Gly Ser  Pro Asp Ile Glu Val  His Thr Tyr
    2045                2050                2055

Pro Phe  Gly Trp Tyr Gln Lys  Pro Ile Ala Gln Asn  Asn Ala Pro
    2060                2065                2070

Ser Phe  Cys Pro Leu Val Val  Leu Pro Ser Leu Thr  Glu Lys Val
    2075                2080                2085

Ser Leu  Asp Ser Trp Gln Ser  Leu Ala Leu Ser Ser  Val Val Val
    2090                2095                2100

Asp Pro  Ser Ile Arg His Phe  Asp Val Ala His Val  Ser Thr Ala
    2105                2110                2115

Ala Thr  Ser Asn Phe Ser Ala  Val Arg Asp Leu Cys  Leu Ser Glu
    2120                2125                2130

Cys Ser  Gln His Glu Ala Cys  Leu Ile Thr Thr Leu  Gln Thr Gln
    2135                2140                2145

Pro Gly  Ala Val Arg Cys Met  Phe Tyr Ala Asp Thr  Gln Ser Cys
    2150                2155                2160

Thr His  Ser Leu Gln Gly Gln  Asn Cys Arg Leu Leu  Leu Arg Glu
    2165                2170                2175

Glu Ala  Thr His Ile Tyr Arg  Lys Pro Gly Ile Ser  Leu Leu Ser
    2180                2185                2190

Tyr Glu  Ala Ser Val Pro Ser  Val Pro Ile Ser Thr  His Gly Arg
    2195                2200                2205

Leu Leu  Gly Arg Ser Gln Ala  Ile Gln Val Gly Thr  Ser Trp Lys
    2210                2215                2220

Gln Val  Asp Gln Phe Leu Gly  Val Pro Tyr Ala Ala  Pro Pro Leu
    2225                2230                2235

Ala Glu  Arg Arg Phe Gln Ala  Pro Glu Pro Leu Asn  Trp Thr Gly
    2240                2245                2250

Ser Trp  Asp Ala Ser Lys Pro  Arg Ala Ser Cys Trp  Gln Pro Gly
    2255                2260                2265

Thr Arg  Thr Ser Thr Ser Pro  Gly Val Ser Glu Asp  Cys Leu Tyr
    2270                2275                2280

Leu Asn  Val Phe Ile Pro Gln  Asn Val Ala Pro Asn  Ala Ser Val
    2285                2290                2295

Leu Val  Phe Phe His Asn Thr  Met Asp Arg Glu Glu  Ser Glu Gly
    2300                2305                2310

Trp Pro  Ala Ile Asp Gly Ser  Phe Leu Ala Ala Val  Gly Asn Leu
    2315                2320                2325

Ile Val  Val Thr Ala Ser Tyr  Arg Val Gly Val Phe  Gly Phe Leu
    2330                2335                2340

Ser Ser  Gly Ser Gly Glu Val  Ser Gly Asn Trp Gly  Leu Leu Asp
    2345                2350                2355

Gln Val  Ala Ala Leu Thr Trp  Val Gln Thr His Ile  Arg Gly Phe
    2360                2365                2370

Gly Gly  Asp Pro Arg Arg Val  Ser Leu Ala Ala Asp  Arg Gly Gly
    2375                2380                2385

Ala Asp  Val Ala Ser Ile His  Leu Leu Thr Ala Arg  Ala Thr Asn
    2390                2395                2400
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Ser Gln  Leu Phe Arg Arg Ala  Val Leu Met Gly Gly  Ser Ala Leu
    2405                 2410                 2415

Ser Pro  Ala Ala Val Ile Ser  His Glu Arg Ala Gln  Gln Gln Ala
    2420                 2425                 2430

Ile Ala  Leu Ala Lys Glu Val  Ser Cys Pro Met Ser  Ser Ser Gln
    2435                 2440                 2445

Glu Val  Val Ser Cys Leu Arg  Gln Lys Pro Ala Asn  Val Leu Asn
    2450                 2455                 2460

Asp Ala  Gln Thr Lys Leu Leu  Ala Val Ser Gly Pro  Phe His Tyr
    2465                 2470                 2475

Trp Gly  Pro Val Ile Asp Gly  His Phe Leu Arg Glu  Pro Pro Ala
    2480                 2485                 2490

Arg Ala  Leu Lys Arg Ser Leu  Trp Val Glu Val Asp  Leu Leu Ile
    2495                 2500                 2505

Gly Ser  Ser Gln Asp Asp Gly  Leu Ile Asn Arg Ala  Lys Ala Val
    2510                 2515                 2520

Lys Gln  Phe Glu Glu Ser Arg  Gly Arg Thr Ser Ser  Lys Thr Ala
    2525                 2530                 2535

Phe Tyr  Gln Ala Leu Gln Asn  Ser Leu Gly Gly Glu  Asp Ser Asp
    2540                 2545                 2550

Ala Arg  Val Glu Ala Ala Ala  Thr Trp Tyr Tyr Ser  Leu Glu His
    2555                 2560                 2565

Ser Thr  Asp Asp Tyr Ala Ser  Phe Ser Arg Ala Leu  Glu Asn Ala
    2570                 2575                 2580

Thr Arg  Asp Tyr Phe Ile Ile  Cys Pro Ile Ile Asp  Met Ala Ser
    2585                 2590                 2595

Ala Trp  Ala Lys Arg Ala Arg  Gly Asn Val Phe Met  Tyr His Ala
    2600                 2605                 2610

Pro Glu  Asn Tyr Gly His Gly  Ser Leu Glu Leu Leu  Ala Asp Val
    2615                 2620                 2625

Gln Phe  Ala Leu Gly Leu Pro  Phe Tyr Pro Ala Tyr  Glu Gly Gln
    2630                 2635                 2640

Phe Ser  Leu Glu Glu Lys Ser  Leu Ser Leu Lys Ile  Met Gln Tyr
    2645                 2650                 2655

Phe Ser  His Phe Ile Arg Ser  Gly Asn Pro Asn Tyr  Pro Tyr Glu
    2660                 2665                 2670

Phe Ser  Arg Lys Val Pro Thr  Phe Ala Thr Pro Trp  Pro Asp Phe
    2675                 2680                 2685

Val Pro  Arg Ala Gly Gly Glu  Asn Tyr Lys Glu Phe  Ser Glu Leu
    2690                 2695                 2700

Leu Pro  Asn Arg Gln Gly Leu  Lys Lys Ala Asp Cys  Ser Phe Trp
    2705                 2710                 2715

Ser Lys  Tyr Ile Ser Ser Leu  Lys Thr Ser Ala Asp  Gly Ala Lys
    2720                 2725                 2730

Gly Gly  Gln Ser Ala Glu Ser  Glu Glu Glu Glu Leu  Thr Ala Gly
    2735                 2740                 2745

Ser Gly  Leu Arg Glu Asp Leu  Leu Ser Leu Gln Glu  Pro Gly Ser
    2750                 2755                 2760

Lys Thr  Tyr Ser Lys
    2765
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<210> SEQ ID NO 2
<211> LENGTH: 2711
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 2

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Met Ala Leu Val Leu Glu Ile Phe Thr Leu Leu Ala Ser Ile Cys Trp
1               5                  10                  15

Val Ser Ala Asn Ile Phe Glu Tyr Gln Val Asp Ala Gln Pro Leu Arg
            20                  25                  30

Pro Cys Glu Leu Gln Arg Glu Thr Ala Phe Leu Lys Gln Ala Asp Tyr
        35                  40                  45

Val Pro Gln Cys Ala Glu Asp Gly Ser Phe Gln Thr Val Gln Cys Gln
    50                  55                  60

Asn Asp Gly Arg Ser Cys Trp Cys Val Gly Ala Asn Gly Ser Glu Val
65                  70                  75                  80

Leu Gly Ser Arg Gln Pro Gly Arg Pro Val Ala Cys Leu Ser Phe Cys
                85                  90                  95

Gln Leu Gln Lys Gln Gln Ile Leu Leu Ser Gly Tyr Ile Asn Ser Thr
            100                 105                 110

Asp Thr Ser Tyr Leu Pro Gln Cys Gln Asp Ser Gly Asp Tyr Ala Pro
        115                 120                 125

Val Gln Cys Asp Val Gln Gln Val Gln Cys Trp Cys Val Asp Ala Glu
    130                 135                 140

Gly Met Glu Val Tyr Gly Thr Arg Gln Leu Gly Arg Pro Lys Arg Cys
145                 150                 155                 160

Pro Arg Ser Cys Glu Ile Arg Asn Arg Arg Leu Leu His Gly Val Gly
                165                 170                 175

Asp Lys Ser Pro Pro Gln Cys Ser Ala Glu Gly Glu Phe Met Pro Val
            180                 185                 190

Gln Cys Lys Phe Val Asn Thr Thr Asp Met Met Ile Phe Asp Leu Val
        195                 200                 205

His Ser Tyr Asn Arg Phe Pro Asp Ala Phe Val Thr Phe Ser Ser Phe
    210                 215                 220

Gln Arg Arg Phe Pro Glu Val Ser Gly Tyr Cys His Cys Ala Asp Ser
225                 230                 235                 240

Gln Gly Arg Glu Leu Ala Glu Thr Gly Leu Glu Leu Leu Leu Asp Glu
                245                 250                 255

Ile Tyr Asp Thr Ile Phe Ala Gly Leu Asp Leu Pro Ser Thr Phe Thr
            260                 265                 270

Glu Thr Thr Leu Tyr Arg Ile Leu Gln Arg Arg Phe Leu Ala Val Gln
        275                 280                 285

Ser Val Ile Ser Gly Arg Phe Arg Cys Pro Thr Lys Cys Glu Val Glu
    290                 295                 300

Arg Phe Thr Ala Thr Ser Phe Gly His Pro Tyr Val Pro Ser Cys Arg
305                 310                 315                 320

Arg Asn Gly Asp Tyr Gln Ala Val Gln Cys Gln Thr Glu Gly Pro Cys
                325                 330                 335

Trp Cys Val Asp Ala Gln Gly Lys Glu Met His Gly Thr Arg Gln Gln
            340                 345                 350

Gly Glu Pro Pro Ser Cys Ala Glu Gly Gln Ser Cys Ala Ser Glu Arg
        355                 360                 365

Gln Gln Ala Leu Ser Arg Leu Tyr Phe Gly Thr Ser Gly Tyr Phe Ser
    370                 375                 380

Gln His Asp Leu Phe Ser Ser Pro Glu Lys Arg Trp Ala Ser Pro Arg
385                 390                 395                 400

Val Ala Arg Phe Ala Thr Ser Cys Pro Pro Thr Ile Lys Glu Leu Phe
                405                 410                 415
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Val Asp Ser Gly Leu Leu Arg Pro Met Val Glu Gly Gln Ser Gln Gln
            420                 425                 430

Phe Ser Val Ser Glu Asn Leu Leu Lys Glu Ala Ile Arg Ala Ile Phe
        435                 440                 445

Pro Ser Arg Gly Leu Ala Arg Leu Ala Leu Gln Phe Thr Thr Asn Pro
    450                 455                 460

Lys Arg Leu Gln Gln Asn Leu Phe Gly Gly Lys Phe Leu Val Asn Val
465                 470                 475                 480

Gly Gln Phe Asn Leu Ser Gly Ala Leu Gly Thr Arg Gly Thr Phe Asn
                485                 490                 495

Phe Ser Gln Phe Phe Gln Gln Leu Gly Leu Ala Ser Phe Leu Asn Gly
            500                 505                 510

Gly Arg Gln Glu Asp Leu Ala Lys Pro Leu Ser Val Gly Leu Asp Ser
        515                 520                 525

Asn Ser Ser Thr Gly Thr Pro Glu Ala Ala Lys Lys Asp Gly Thr Met
    530                 535                 540

Asn Lys Pro Thr Val Gly Ser Phe Gly Phe Glu Ile Asn Leu Gln Glu
545                 550                 555                 560

Asn Gln Asn Ala Leu Lys Phe Leu Ala Ser Leu Leu Glu Leu Pro Glu
                565                 570                 575

Phe Leu Leu Phe Leu Gln His Ala Ile Ser Val Pro Glu Asp Val Ala
            580                 585                 590

Arg Asp Leu Gly Asp Val Met Glu Thr Val Leu Ser Ser Gln Thr Cys
        595                 600                 605

Glu Gln Thr Pro Glu Arg Leu Phe Val Pro Ser Cys Thr Thr Glu Gly
    610                 615                 620

Ser Tyr Glu Asp Val Gln Cys Phe Ser Gly Glu Cys Trp Cys Val Asn
625                 630                 635                 640

Ser Trp Gly Lys Glu Leu Pro Gly Ser Arg Val Arg Gly Gly Gln Pro
                645                 650                 655

Arg Cys Pro Thr Asp Cys Glu Lys Gln Arg Ala Arg Met Gln Ser Leu
            660                 665                 670

Met Gly Ser Gln Pro Ala Gly Ser Thr Leu Phe Val Pro Ala Cys Thr
        675                 680                 685

Ser Glu Gly His Phe Leu Pro Val Gln Cys Phe Asn Ser Glu Cys Tyr
    690                 695                 700

Cys Val Asp Ala Glu Gly Gln Ala Ile Pro Gly Thr Arg Ser Ala Ile
705                 710                 715                 720

Gly Lys Pro Lys Lys Cys Pro Thr Pro Cys Gln Leu Gln Ser Glu Gln
                725                 730                 735

Ala Phe Leu Arg Thr Val Gln Ala Leu Leu Ser Asn Ser Ser Met Leu
            740                 745                 750

Pro Thr Leu Ser Asp Thr Tyr Ile Pro Gln Cys Ser Thr Asp Gly Gln
        755                 760                 765

Trp Arg Gln Val Gln Cys Asn Gly Pro Pro Glu Gln Val Phe Glu Leu
    770                 775                 780

Tyr Gln Arg Trp Glu Ala Gln Asn Lys Gly Gln Asp Leu Thr Pro Ala
785                 790                 795                 800

Lys Leu Leu Val Lys Ile Met Ser Tyr Arg Glu Ala Ala Ser Gly Asn
                805                 810                 815

Phe Ser Leu Phe Ile Gln Ser Leu Tyr Glu Ala Gly Gln Gln Asp Val
            820                 825                 830

Phe Pro Val Leu Ser Gln Tyr Pro Ser Leu Gln Asp Val Pro Leu Ala
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        835                 840                 845

Ala Leu Glu Gly Lys Arg Pro Gln Pro Arg Glu Asn Ile Leu Leu Glu
    850                 855                 860

Pro Tyr Leu Phe Trp Gln Ile Leu Asn Gly Gln Leu Ser Gln Tyr Pro
865                 870                 875                 880

Gly Ser Tyr Ser Asp Phe Ser Thr Pro Leu Ala His Phe Asp Leu Arg
                885                 890                 895

Asn Cys Trp Cys Val Asp Glu Ala Gly Gln Glu Leu Glu Gly Met Arg
            900                 905                 910

Ser Glu Pro Ser Lys Leu Pro Thr Cys Pro Gly Ser Cys Glu Glu Ala
        915                 920                 925

Lys Leu Arg Val Leu Gln Phe Ile Arg Glu Thr Glu Glu Ile Val Ser
    930                 935                 940

Ala Ser Asn Ser Ser Arg Phe Pro Leu Gly Glu Ser Phe Leu Val Ala
945                 950                 955                 960

Lys Gly Ile Arg Leu Arg Asn Glu Asp Leu Gly Leu Pro Pro Leu Phe
                965                 970                 975

Pro Pro Arg Glu Ala Phe Ala Glu Gln Phe Leu Arg Gly Ser Asp Tyr
            980                 985                 990

Ala Ile Arg Leu Ala Ala Gln Ser  Thr Leu Ser Phe Tyr  Gln Arg Arg
        995                 1000                1005

Arg Phe  Ser Pro Asp Asp Ser  Ala Gly Ala Ser Ala  Leu Leu Arg
    1010                1015                1020

Ser Gly  Pro Tyr Met Pro Gln  Cys Asp Ala Phe Gly  Ser Trp Glu
    1025                1030                1035

Pro Val  Gln Cys His Ala Gly  Thr Gly His Cys Trp  Cys Val Asp
    1040                1045                1050

Glu Lys  Gly Gly Phe Ile Pro  Gly Ser Leu Thr Ala  Arg Ser Leu
    1055                1060                1065

Gln Ile  Pro Gln Cys Pro Thr  Thr Cys Glu Lys Ser  Arg Thr Ser
    1070                1075                1080

Gly Leu  Leu Ser Ser Trp Lys  Gln Ala Arg Ser Gln  Glu Asn Pro
    1085                1090                1095

Ser Pro  Lys Asp Leu Phe Val  Pro Ala Cys Leu Glu  Thr Gly Glu
    1100                1105                1110

Tyr Ala  Arg Leu Gln Ala Ser  Gly Ala Gly Thr Trp  Cys Val Asp
    1115                1120                1125

Pro Ala  Ser Gly Glu Glu Leu  Arg Pro Gly Ser Ser  Ser Ser Ala
    1130                1135                1140

Gln Cys  Pro Ser Leu Cys Asn  Val Leu Lys Ser Gly  Val Leu Ser
    1145                1150                1155

Arg Arg  Val Ser Pro Gly Tyr  Val Pro Ala Cys Arg  Ala Glu Asp
    1160                1165                1170

Gly Gly  Phe Ser Pro Val Gln  Cys Asp Gln Ala Gln  Gly Ser Cys
    1175                1180                1185

Trp Cys  Val Met Asp Ser Gly  Glu Glu Val Pro Gly  Thr Arg Val
    1190                1195                1200

Thr Gly  Gly Gln Pro Ala Cys  Glu Ser Pro Arg Cys  Pro Leu Pro
    1205                1210                1215

Phe Asn  Ala Ser Glu Val Val  Gly Gly Thr Ile Leu  Cys Glu Thr
    1220                1225                1230

Ile Ser  Gly Pro Thr Gly Ser  Ala Met Gln Gln Cys  Gln Leu Leu
    1235                1240                1245
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Cys Arg  Gln Gly Ser Trp Ser  Val Phe Pro Pro Gly  Pro Leu Ile
    1250                 1255                 1260

Cys Ser  Leu Glu Ser Gly Arg  Trp Glu Ser Gln Leu  Pro Gln Pro
    1265                 1270                 1275

Arg Ala  Cys Gln Arg Pro Gln  Leu Trp Gln Thr Ile  Gln Thr Gln
    1280                 1285                 1290

Gly His  Phe Gln Leu Gln Leu  Pro Pro Gly Lys Met  Cys Ser Ala
    1295                 1300                 1305

Asp Tyr  Ala Asp Leu Leu Gln  Thr Phe Gln Val Phe  Ile Leu Asp
    1310                 1315                 1320

Glu Leu  Thr Ala Arg Gly Phe  Cys Gln Ile Gln Val  Lys Thr Phe
    1325                 1330                 1335

Gly Thr  Leu Val Ser Ile Pro  Val Cys Asn Asn Ser  Ser Val Gln
    1340                 1345                 1350

Val Gly  Cys Leu Thr Arg Glu  Arg Leu Gly Val Asn  Val Thr Trp
    1355                 1360                 1365

Lys Ser  Arg Leu Glu Asp Ile  Pro Val Ala Ser Leu  Pro Asp Leu
    1370                 1375                 1380

His Asp  Ile Glu Arg Ala Leu  Val Gly Lys Asp Leu  Leu Gly Arg
    1385                 1390                 1395

Phe Thr  Asp Leu Ile Gln Ser  Gly Ser Phe Gln Leu  His Leu Asp
    1400                 1405                 1410

Ser Lys  Thr Phe Pro Ala Glu  Thr Ile Arg Phe Leu  Gln Gly Asp
    1415                 1420                 1425

His Phe  Gly Thr Ser Pro Arg  Thr Trp Phe Gly Cys  Ser Glu Gly
    1430                 1435                 1440

Phe Tyr  Gln Val Leu Thr Ser  Glu Ala Ser Gln Asp  Gly Leu Gly
    1445                 1450                 1455

Cys Val  Lys Cys Pro Glu Gly  Ser Tyr Ser Gln Asp  Glu Glu Cys
    1460                 1465                 1470

Ile Pro  Cys Pro Val Gly Phe  Tyr Gln Glu Gln Ala  Gly Ser Leu
    1475                 1480                 1485

Ala Cys  Val Pro Cys Pro Val  Gly Arg Thr Thr Ile  Ser Ala Gly
    1490                 1495                 1500

Ala Phe  Ser Gln Thr His Leu  Met Gln Lys Phe Glu  Lys Val Pro
    1505                 1510                 1515

Glu Ser  Lys Val Ile Phe Asp  Ala Asn Ala Pro Val  Ala Val Arg
    1520                 1525                 1530

Ser Lys  Val Pro Asp Ser Glu  Phe Pro Val Met Gln  Cys Leu Thr
    1535                 1540                 1545

Asp Cys  Thr Glu Asp Glu Ala  Cys Ser Phe Phe Thr  Val Ser Thr
    1550                 1555                 1560

Thr Glu  Pro Glu Ile Ser Cys  Asp Phe Tyr Ala Trp  Thr Ser Asp
    1565                 1570                 1575

Asn Val  Ala Cys Met Thr Ser  Asp Gln Lys Arg Asp  Ala Leu Gly
    1580                 1585                 1590

Asn Ser  Lys Ala Thr Ser Phe  Gly Ser Leu Arg Cys  Gln Val Lys
    1595                 1600                 1605

Val Arg  Ser His Gly Gln Asp  Ser Pro Ala Val Tyr  Leu Lys Lys
    1610                 1615                 1620

Gly Gln  Gly Ser Thr Thr Thr  Leu Gln Lys Arg Phe  Glu Pro Thr
    1625                 1630                 1635

Gly Phe  Gln Asn Met Leu Ser  Gly Leu Tyr Asn Pro  Ile Val Phe
    1640                 1645                 1650
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Ser Ala  Ser Gly Ala Asn Leu  Thr Asp Ala His Leu  Phe Cys Leu
    1655                 1660                 1665

Leu Ala  Cys Asp Arg Asp Leu  Cys Cys Asp Gly Phe  Val Leu Thr
    1670                 1675                 1680

Gln Val  Gln Gly Gly Ala Ile  Ile Cys Gly Leu Leu  Ser Ser Pro
    1685                 1690                 1695

Ser Val  Leu Leu Cys Asn Val  Lys Asp Trp Met Asp  Pro Ser Glu
    1700                 1705                 1710

Ala Trp  Ala Asn Ala Thr Cys  Pro Gly Val Thr Tyr  Asp Gln Glu
    1715                 1720                 1725

Ser His  Gln Val Ile Leu Arg  Leu Gly Asp Gln Glu  Phe Ile Lys
    1730                 1735                 1740

Ser Leu  Thr Pro Leu Glu Gly  Thr Gln Asp Thr Phe  Thr Asn Phe
    1745                 1750                 1755

Gln Gln  Val Tyr Leu Trp Lys  Asp Ser Asp Met Gly  Ser Arg Pro
    1760                 1765                 1770

Glu Ser  Met Gly Cys Arg Lys  Asp Thr Val Pro Arg  Pro Ala Ser
    1775                 1780                 1785

Pro Thr  Glu Ala Gly Leu Thr  Thr Glu Leu Phe Ser  Pro Val Asp
    1790                 1795                 1800

Leu Asn  Gln Val Ile Val Asn  Gly Asn Gln Ser Leu  Ser Ser Gln
    1805                 1810                 1815

Lys His  Trp Leu Phe Lys His  Leu Phe Ser Ala Gln  Gln Ala Asn
    1820                 1825                 1830

Leu Trp  Cys Leu Ser Arg Cys  Val Gln Glu His Ser  Phe Cys Gln
    1835                 1840                 1845

Leu Ala  Glu Ile Thr Glu Ser  Ala Ser Leu Tyr Phe  Thr Cys Thr
    1850                 1855                 1860

Leu Tyr  Pro Glu Ala Gln Val  Cys Asp Asp Ile Met  Glu Ser Asn
    1865                 1870                 1875

Ala Gln  Gly Cys Arg Leu Ile  Leu Pro Gln Met Pro  Lys Ala Leu
    1880                 1885                 1890

Phe Arg  Lys Lys Val Ile Leu  Glu Asp Lys Val Lys  Asn Phe Tyr
    1895                 1900                 1905

Thr Arg  Leu Pro Phe Gln Lys  Leu Met Gly Ile Ser  Ile Arg Asn
    1910                 1915                 1920

Lys Val  Pro Met Ser Glu Lys  Ser Ile Ser Asn Gly  Phe Phe Glu
    1925                 1930                 1935

Cys Glu  Arg Arg Cys Asp Ala  Asp Pro Cys Cys Thr  Gly Phe Gly
    1940                 1945                 1950

Phe Leu  Asn Val Ser Gln Leu  Lys Gly Gly Glu Val  Thr Cys Leu
    1955                 1960                 1965

Thr Leu  Asn Ser Leu Gly Ile  Gln Met Cys Ser Glu  Glu Asn Gly
    1970                 1975                 1980

Gly Ala  Trp Arg Ile Leu Asp  Cys Gly Ser Pro Asp  Ile Glu Val
    1985                 1990                 1995

His Thr  Tyr Pro Phe Gly Trp  Tyr Gln Lys Pro Ile  Ala Gln Asn
    2000                 2005                 2010

Asn Ala  Pro Ser Phe Cys Pro  Leu Val Val Leu Pro  Ser Leu Thr
    2015                 2020                 2025

Glu Lys  Val Ser Leu Asp Ser  Trp Gln Ser Leu Ala  Leu Ser Ser
    2030                 2035                 2040

Val Val  Val Asp Pro Ser Ile  Arg His Phe Asp Val  Ala His Val
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    2045                2050                2055

Ser Thr  Ala Ala Thr Ser Asn  Phe Ser Ala Val Arg  Asp Leu Cys
    2060                2065                2070

Leu Ser  Glu Cys Ser Gln His  Glu Ala Cys Leu Ile  Thr Thr Leu
    2075                2080                2085

Gln Thr  Gln Pro Gly Ala Val  Arg Cys Met Phe Tyr  Ala Asp Thr
    2090                2095                2100

Gln Ser  Cys Thr His Ser Leu  Gln Gly Gln Asn Cys  Arg Leu Leu
    2105                2110                2115

Leu Arg  Glu Glu Ala Thr His  Ile Tyr Arg Lys Pro  Gly Ile Ser
    2120                2125                2130

Leu Leu  Ser Tyr Glu Ala Ser  Val Pro Ser Val Pro  Ile Ser Thr
    2135                2140                2145

His Gly  Arg Leu Leu Gly Arg  Ser Gln Ala Ile Gln  Val Gly Thr
    2150                2155                2160

Ser Trp  Lys Gln Val Asp Gln  Phe Leu Gly Val Pro  Tyr Ala Ala
    2165                2170                2175

Pro Pro  Leu Ala Glu Arg Arg  Phe Gln Ala Pro Glu  Pro Leu Asn
    2180                2185                2190

Trp Thr  Gly Ser Trp Asp Ala  Ser Lys Pro Arg Ala  Ser Cys Trp
    2195                2200                2205

Gln Pro  Gly Thr Arg Thr Ser  Thr Ser Pro Gly Val  Ser Glu Asp
    2210                2215                2220

Cys Leu  Tyr Leu Asn Val Phe  Ile Pro Gln Asn Val  Ala Pro Asn
    2225                2230                2235

Ala Ser  Val Leu Val Phe Phe  His Asn Thr Met Asp  Arg Glu Glu
    2240                2245                2250

Ser Glu  Gly Trp Pro Ala Ile  Asp Gly Ser Phe Leu  Ala Ala Val
    2255                2260                2265

Gly Asn  Leu Ile Val Val Thr  Ala Ser Tyr Arg Val  Gly Val Phe
    2270                2275                2280

Gly Phe  Leu Ser Ser Gly Ser  Gly Glu Val Ser Gly  Asn Trp Gly
    2285                2290                2295

Leu Leu  Asp Gln Val Ala Ala  Leu Thr Trp Val Gln  Thr His Ile
    2300                2305                2310

Arg Gly  Phe Gly Gly Asp Pro  Arg Arg Val Ser Leu  Ala Ala Asp
    2315                2320                2325

Arg Gly  Gly Ala Asp Val Ala  Ser Ile His Leu Leu  Thr Ala Arg
    2330                2335                2340

Ala Thr  Asn Ser Gln Leu Phe  Arg Arg Ala Val Leu  Met Gly Gly
    2345                2350                2355

Ser Ala  Leu Ser Pro Ala Ala  Val Ile Ser His Glu  Arg Ala Gln
    2360                2365                2370

Gln Gln  Ala Ile Ala Leu Ala  Lys Glu Val Ser Cys  Pro Met Ser
    2375                2380                2385

Ser Ser  Gln Glu Val Val Ser  Cys Leu Arg Gln Lys  Pro Ala Asn
    2390                2395                2400

Val Leu  Asn Asp Ala Gln Thr  Lys Leu Leu Ala Val  Ser Gly Pro
    2405                2410                2415

Phe His  Tyr Trp Gly Pro Val  Ile Asp Gly His Phe  Leu Arg Glu
    2420                2425                2430

Pro Pro  Ala Arg Ala Leu Lys  Arg Ser Leu Trp Val  Glu Val Asp
    2435                2440                2445
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Leu Leu  Ile Gly Ser Ser Gln  Asp Asp Gly Leu Ile  Asn Arg Ala
    2450                 2455                 2460

Lys Ala  Val Lys Gln Phe Glu  Glu Ser Arg Gly Arg  Thr Ser Ser
    2465                 2470                 2475

Lys Thr  Ala Phe Tyr Gln Ala  Leu Gln Asn Ser Leu  Gly Gly Glu
    2480                 2485                 2490

Asp Ser  Asp Ala Arg Val Glu  Ala Ala Ala Thr Trp  Tyr Tyr Ser
    2495                 2500                 2505

Leu Glu  His Ser Thr Asp Asp  Tyr Ala Ser Phe Ser  Arg Ala Leu
    2510                 2515                 2520

Glu Asn  Ala Thr Arg Asp Tyr  Phe Ile Ile Cys Pro  Ile Ile Asp
    2525                 2530                 2535

Met Ala  Ser Ala Trp Ala Lys  Arg Ala Arg Gly Asn  Val Phe Met
    2540                 2545                 2550

Tyr His  Ala Pro Glu Asn Tyr  Gly His Gly Ser Leu  Glu Leu Leu
    2555                 2560                 2565

Ala Asp  Val Gln Phe Ala Leu  Gly Leu Pro Phe Tyr  Pro Ala Tyr
    2570                 2575                 2580

Glu Gly  Gln Phe Ser Leu Glu  Glu Lys Ser Leu Ser  Leu Lys Ile
    2585                 2590                 2595

Met Gln  Tyr Phe Ser His Phe  Ile Arg Ser Gly Asn  Pro Asn Tyr
    2600                 2605                 2610

Pro Tyr  Glu Phe Ser Arg Lys  Val Pro Thr Phe Ala  Thr Pro Trp
    2615                 2620                 2625

Pro Asp  Phe Val Pro Arg Ala  Gly Gly Glu Asn Tyr  Lys Glu Phe
    2630                 2635                 2640

Ser Glu  Leu Leu Pro Asn Arg  Gln Gly Leu Lys Lys  Ala Asp Cys
    2645                 2650                 2655

Ser Phe  Trp Ser Lys Tyr Ile  Ser Ser Leu Lys Thr  Ser Ala Asp
    2660                 2665                 2670

Gly Ala  Lys Gly Gly Gln Ser  Ala Glu Ser Glu Glu  Glu Glu Leu
    2675                 2680                 2685

Thr Ala  Gly Ser Gly Leu Arg  Glu Asp Leu Leu Ser  Leu Gln Glu
    2690                 2695                 2700

Pro Gly  Ser Lys Thr Tyr Ser  Lys
    2705                 2710
```

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<210> SEQ ID NO 3
<211> LENGTH: 1574
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Ile Pro Arg Lys Pro Ile Ser Lys Arg Pro Val Arg Pro Ser Leu Pro
1               5                  10                  15

Arg Ser Pro Arg Cys Pro Leu Pro Phe Asn Ala Ser Glu Val Val Gly
            20                  25                  30

Gly Thr Ile Leu Cys Glu Thr Ile Ser Gly Pro Thr Gly Ser Ala Met
        35                  40                  45

Gln Gln Cys Gln Leu Leu Cys Arg Gln Gly Ser Trp Ser Val Phe Pro
    50                  55                  60

Pro Gly Pro Leu Ile Cys Ser Leu Glu Ser Gly Arg Trp Glu Ser Gln
65                  70                  75                  80

Leu Pro Gln Pro Arg Ala Cys Gln Arg Pro Gln Leu Trp Gln Thr Ile
                85                  90                  95
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Gln Thr Gln Gly His Phe Gln Leu Gln Leu Pro Pro Gly Lys Met Cys
            100                 105                 110

Ser Ala Asp Tyr Ala Gly Leu Leu Gln Thr Phe Gln Val Phe Ile Leu
        115                 120                 125

Asp Glu Leu Thr Ala Arg Gly Phe Cys Gln Ile Gln Val Lys Thr Phe
    130                 135                 140

Gly Thr Leu Val Ser Ile Pro Val Cys Asn Asn Ser Ser Val Gln Val
145                 150                 155                 160

Gly Cys Leu Thr Arg Glu Arg Leu Gly Val Asn Val Thr Trp Lys Ser
                165                 170                 175

Arg Leu Glu Asp Ile Pro Val Ala Ser Leu Pro Asp Leu His Asp Ile
            180                 185                 190

Glu Arg Ala Leu Val Gly Lys Asp Leu Leu Gly Arg Phe Thr Asp Leu
        195                 200                 205

Ile Gln Ser Gly Ser Phe Gln Leu His Leu Asp Ser Lys Thr Phe Pro
    210                 215                 220

Ala Glu Thr Ile Arg Phe Leu Gln Gly Asp His Phe Gly Thr Ser Pro
225                 230                 235                 240

Arg Thr Trp Phe Gly Cys Ser Glu Gly Phe Tyr Gln Val Leu Thr Ser
                245                 250                 255

Glu Ala Ser Gln Asp Gly Leu Gly Cys Val Lys Cys Pro Glu Gly Ser
            260                 265                 270

Tyr Ser Gln Asp Glu Glu Cys Ile Pro Cys Pro Val Gly Phe Tyr Gln
        275                 280                 285

Glu Gln Ala Gly Ser Leu Ala Cys Val Pro Cys Pro Val Gly Arg Thr
    290                 295                 300

Thr Ile Ser Ala Gly Ala Phe Ser Gln Thr His Cys Val Thr Asp Cys
305                 310                 315                 320

Gln Arg Asn Glu Ala Gly Leu Gln Cys Asp Gln Asn Gly Gln Tyr Arg
                325                 330                 335

Ala Ser Gln Lys Asp Arg Gly Ser Gly Lys Ala Phe Cys Val Asp Gly
            340                 345                 350

Glu Gly Arg Arg Leu Pro Trp Trp Glu Thr Glu Ala Pro Leu Glu Asp
        355                 360                 365

Ser Gln Cys Leu Met Met Gln Lys Phe Glu Lys Val Pro Glu Ser Lys
    370                 375                 380

Val Ile Phe Asp Ala Asn Ala Pro Val Ala Val Arg Ser Lys Val Pro
385                 390                 395                 400

Asp Ser Glu Phe Pro Val Met Gln Cys Leu Thr Asp Cys Thr Glu Asp
                405                 410                 415

Glu Ala Cys Ser Phe Phe Thr Val Ser Thr Thr Glu Pro Glu Ile Ser
            420                 425                 430

Cys Asp Phe Tyr Ala Trp Thr Ser Asp Asn Val Ala Cys Met Thr Ser
        435                 440                 445

Asp Gln Lys Arg Asp Ala Leu Gly Asn Ser Lys Ala Thr Ser Phe Gly
    450                 455                 460

Ser Leu Arg Cys Gln Val Lys Val Arg Ser His Gly Gln Asp Ser Pro
465                 470                 475                 480

Ala Val Tyr Leu Lys Lys Gly Gln Gly Ser Thr Thr Thr Leu Gln Lys
                485                 490                 495

Arg Phe Glu Pro Thr Gly Phe Gln Asn Met Leu Ser Gly Leu Tyr Asn
            500                 505                 510

Pro Ile Val Phe Ser Ala Ser Gly Ala Asn Leu Thr Asp Ala His Leu
        515                 520                 525
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Phe Cys Leu Leu Ala Cys Asp Arg Asp Leu Cys Cys Asp Gly Phe Val
    530                 535                 540

Leu Thr Gln Val Gln Gly Gly Ala Ile Ile Cys Gly Leu Leu Ser Ser
545                 550                 555                 560

Pro Ser Val Leu Leu Cys Asn Val Lys Asp Trp Met Asp Pro Ser Glu
                565                 570                 575

Ala Trp Ala Asn Ala Thr Cys Pro Gly Val Thr Tyr Asp Gln Glu Ser
            580                 585                 590

His Gln Val Ile Leu Arg Leu Gly Asp Gln Glu Phe Ile Lys Ser Leu
        595                 600                 605

Thr Pro Leu Glu Gly Thr Gln Asp Thr Phe Thr Asn Phe Gln Gln Val
    610                 615                 620

Tyr Leu Trp Lys Asp Ser Asp Met Gly Ser Arg Pro Glu Ser Met Gly
625                 630                 635                 640

Cys Arg Lys Asn Thr Val Pro Arg Pro Ala Ser Pro Thr Glu Ala Gly
                645                 650                 655

Leu Thr Thr Glu Leu Phe Ser Pro Val Asp Leu Asn Gln Val Ile Val
            660                 665                 670

Asn Gly Asn Gln Ser Leu Ser Ser Gln Lys His Trp Leu Phe Lys His
        675                 680                 685

Leu Phe Ser Ala Gln Gln Ala Asn Leu Trp Cys Leu Ser Arg Cys Val
    690                 695                 700

Gln Glu His Ser Phe Cys Gln Leu Ala Glu Ile Thr Glu Ser Ala Ser
705                 710                 715                 720

Leu Tyr Phe Thr Cys Thr Leu Tyr Pro Glu Ala Gln Val Cys Asp Asp
                725                 730                 735

Ile Met Glu Ser Asn Ala Gln Gly Cys Arg Leu Ile Leu Pro Gln Met
            740                 745                 750

Pro Lys Ala Leu Phe Arg Lys Lys Val Ile Leu Glu Asp Lys Val Lys
        755                 760                 765

Asn Phe Tyr Thr Arg Leu Pro Phe Gln Lys Leu Thr Gly Ile Ser Ile
    770                 775                 780

Arg Asn Lys Val Pro Met Ser Glu Lys Ser Ile Ser Asn Gly Phe Phe
785                 790                 795                 800

Glu Cys Glu Arg Arg Cys Asp Ala Asp Pro Cys Cys Thr Gly Phe Gly
                805                 810                 815

Phe Leu Asn Val Ser Gln Leu Lys Gly Gly Glu Val Thr Cys Leu Thr
            820                 825                 830

Leu Asn Ser Leu Gly Ile Gln Met Cys Ser Glu Glu Asn Gly Gly Ala
        835                 840                 845

Trp Arg Ile Leu Asp Cys Gly Ser Pro Asp Ile Glu Val His Thr Tyr
    850                 855                 860

Pro Phe Gly Trp Tyr Gln Lys Pro Ile Ala Gln Asn Asn Ala Pro Ser
865                 870                 875                 880

Phe Cys Pro Leu Val Val Leu Pro Ser Leu Thr Glu Lys Val Ser Leu
                885                 890                 895

Asp Ser Trp Gln Ser Leu Ala Leu Ser Ser Val Val Val Asp Pro Ser
            900                 905                 910

Ile Arg His Phe Asp Val Ala His Val Ser Thr Ala Ala Thr Ser Asn
        915                 920                 925

Phe Ser Ala Val Arg Asp Leu Cys Leu Ser Glu Cys Ser Gln His Glu
    930                 935                 940

Ala Cys Leu Ile Thr Thr Leu Gln Thr Gln Pro Gly Ala Val Arg Cys
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945                 950                 955                 960

Met Phe Tyr Ala Asp Thr Gln Ser Cys Thr His Ser Leu Gln Gly Gln
                965                 970                 975

Asn Cys Arg Leu Leu Leu Arg Glu Glu Ala Thr His Ile Tyr Arg Lys
            980                 985                 990

Pro Gly Ile Ser Leu Leu Ser Tyr  Glu Ala Ser Val Pro  Ser Val Pro
        995                 1000                1005

Ile Ser  Thr His Gly Arg Leu  Leu Gly Arg Ser Gln  Ala Ile Gln
    1010                1015                1020

Val Gly  Thr Ser Trp Lys Gln  Val Asp Gln Phe Leu  Gly Val Pro
    1025                1030                1035

Tyr Ala  Ala Pro Pro Leu Ala  Glu Arg Arg Phe Gln  Ala Pro Glu
    1040                1045                1050

Pro Leu  Asn Trp Thr Gly Ser  Trp Asp Ala Ser Lys  Pro Arg Ala
    1055                1060                1065

Ser Cys  Trp Gln Pro Gly Thr  Arg Thr Ser Thr Ser  Pro Gly Val
    1070                1075                1080

Ser Glu  Asp Cys Leu Tyr Leu  Asn Val Phe Ile Pro  Gln Asn Val
    1085                1090                1095

Ala Pro  Asn Ala Ser Val Leu  Val Phe Phe His Asn  Thr Met Asp
    1100                1105                1110

Arg Glu  Glu Ser Glu Gly Trp  Pro Ala Ile Asp Gly  Ser Phe Leu
    1115                1120                1125

Ala Ala  Val Gly Asn Leu Ile  Val Val Thr Ala Ser  Tyr Arg Val
    1130                1135                1140

Gly Val  Phe Gly Phe Leu Ser  Ser Gly Ser Gly Glu  Val Ser Gly
    1145                1150                1155

Asn Trp  Gly Leu Leu Asp Gln  Val Ala Ala Leu Thr  Trp Val Gln
    1160                1165                1170

Thr His  Ile Arg Gly Phe Gly  Gly Asp Pro Arg Arg  Val Ser Leu
    1175                1180                1185

Ala Ala  Asp Arg Gly Gly Ala  Asp Val Ala Ser Ile  His Leu Leu
    1190                1195                1200

Thr Ala  Arg Ala Thr Asn Ser  Gln Leu Phe Arg Arg  Ala Val Leu
    1205                1210                1215

Met Gly  Gly Ser Ala Leu Ser  Pro Ala Ala Val Ile  Ser His Glu
    1220                1225                1230

Arg Ala  Gln Gln Gln Ala Ile  Ala Leu Ala Lys Glu  Val Ser Cys
    1235                1240                1245

Pro Met  Ser Ser Ser Gln Glu  Val Val Ser Cys Leu  Arg Gln Lys
    1250                1255                1260

Pro Ala  Asn Val Leu Asn Asp  Ala Gln Thr Lys Leu  Leu Ala Val
    1265                1270                1275

Ser Gly  Pro Phe His Tyr Trp  Gly Pro Val Ile Asp  Gly His Phe
    1280                1285                1290

Leu Arg  Glu Pro Pro Ala Arg  Ala Leu Lys Arg Ser  Leu Trp Val
    1295                1300                1305

Glu Val  Asp Leu Leu Ile Gly  Ser Ser Gln Asp Asp  Gly Leu Ile
    1310                1315                1320

Asn Arg  Ala Lys Ala Val Lys  Gln Phe Glu Glu Ser  Gln Gly Arg
    1325                1330                1335

Thr Ser  Ser Lys Thr Ala Phe  Tyr Gln Ala Leu Gln  Asn Ser Leu
    1340                1345                1350
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Gly Gly  Glu Asp Ser Asp Ala  Arg Val Glu Ala Ala  Ala Thr Trp
    1355                 1360                 1365

Tyr Tyr  Ser Leu Glu His Ser  Thr Asp Asp Tyr Ala  Ser Phe Ser
    1370                 1375                 1380

Arg Ala  Leu Glu Asn Ala Thr  Arg Asp Tyr Phe Ile  Ile Cys Pro
    1385                 1390                 1395

Ile Ile  Asp Met Ala Ser Ala  Trp Ala Lys Arg Ala  Arg Gly Asn
    1400                 1405                 1410

Val Phe  Met Tyr His Ala Pro  Glu Asn Tyr Gly His  Gly Ser Leu
    1415                 1420                 1425

Glu Leu  Leu Ala Asp Val Gln  Phe Ala Leu Gly Leu  Pro Phe Tyr
    1430                 1435                 1440

Pro Ala  Tyr Glu Gly Gln Phe  Ser Leu Glu Glu Lys  Ser Leu Ser
    1445                 1450                 1455

Leu Lys  Ile Met Gln Tyr Phe  Ser His Phe Ile Arg  Ser Gly Asn
    1460                 1465                 1470

Pro Asn  Tyr Pro Tyr Glu Phe  Ser Arg Lys Val Pro  Thr Phe Ala
    1475                 1480                 1485

Thr Pro  Trp Pro Asp Phe Val  Pro Arg Ala Gly Gly  Glu Asn Tyr
    1490                 1495                 1500

Lys Glu  Phe Ser Glu Leu Leu  Pro Asn Arg Gln Gly  Leu Lys Lys
    1505                 1510                 1515

Ala Asp  Cys Ser Phe Trp Ser  Lys Tyr Ile Ser Ser  Leu Lys Thr
    1520                 1525                 1530

Ser Ala  Asp Gly Ala Lys Gly  Gly Gln Ser Ala Glu  Ser Glu Glu
    1535                 1540                 1545

Glu Glu  Leu Thr Ala Gly Ser  Gly Leu Arg Glu Asp  Leu Leu Ser
    1550                 1555                 1560

Leu Gln  Glu Pro Gly Ser Lys  Thr Tyr Ser Lys
    1565                 1570


<210> SEQ ID NO 4
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Val Ile Phe Asp Ala Asn Ala Pro Val Ala Val Arg
1               5                   10
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That which is claimed is:

1. A method for determining the amount of thyroglobulin in a test sample, comprising:

(a) digesting thyroglobulin from said test sample to form peptide T129;

(b) purifying said peptide T129 from step (a);

(c) ionizing said peptide T129 from step (b) to generate a multiply charged ion detectable by mass spectrometry;

(d) detecting the amount of one or more ions by mass spectrometry; wherein the amount of said ion(s) detected is related to the amount of said thyroglobulin in said test sample.

2. The method of claim **1**, wherein said ion of step (c) has a mass/charge ratio of 636.4±0.5.

3. The method of claim **1**, wherein one or more of said ions detected in step (d) are selected from the group consisting of ions with m/z of 541.3±0.5, 612.3±0.5, 636.4±0.5, 726.4±0.5, 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

4. The method of claim **1**, wherein one or more of said ions detected in step (d) are selected from the group consisting of ions with m/z of 636.4±0.5, 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

5. The method of claim **1**, wherein said mass spectrometry comprises tandem mass spectrometry.

6. The method of claim **5**, wherein said ionizing of step (c) comprises:

ionizing said peptide T129 from step (b) to generate a multiply charged peptide T129 precursor ion detectable by mass spectrometry;

and colliding said peptide T129 precursor ion with a collision gas to generate one or more peptide T129 fragment ions.

7. The method of claim **6**, wherein said one or more peptide T129 fragment ions are selected from the group consisting of ions with m/z of 541.3±0.5, 612.3±0.5, 726.4±0.5, 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

8. The method of claim 6, wherein said one or more peptide T129 fragment ions are selected from the group consisting of ions with m/z of 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

9. The method of claim 6, wherein said ions detected in step (d) comprise one or more peptide T129 fragment ions.

10. The method of claim 1, wherein step (b) is accomplished by utilizing at least one size separation technique.

11. The method of claim 1, further comprising purifying thyroglobulin from a body fluid or tissue sample to generate a thyroglobulin containing test sample.

12. The method of claim 11, wherein said body fluid or tissue sample comprises plasma or serum.

13. The method of claim 1, wherein said test sample comprises a body fluid.

14. The method of claim 13, wherein said test sample comprises plasma or serum.

15. A method for determining persistence of thyroid tissue in a patient after surgical removal of the thyroid gland, the method comprising:

(a) providing a body fluid or tissue sample obtained from a patient;

(b) processing said sample to generate a processed sample, wherein at least one step in said processing comprises subjecting said processed sample to trypsin under conditions suitable to digest thyroglobulin and thereby generate peptide T129, and at lease a second step in said processing comprises subjecting the post-digested processed sample to one or more purification steps under conditions suitable to enrich peptide T129;

(c) subjecting the processed sample of step (b) to an ionization source under conditions suitable to generate a multiply charged peptide T129 ion detectable by mass spectrometry;

(d) detecting the presence of one or more peptide T129 ions by mass spectrometry;

wherein the presence of said one or more peptide T129 ions indicates persistence of thyroid tissue in the patient.

16. The method of claim 15, wherein said ion of step (c) has a mass/charge ratio of 636.4±0.5.

17. The method of claim 15, wherein one or more of said ions detected in step (d) are selected from the group consisting of ions with m/z of 541.3±0.5, 612.3±0.5, 636.4±0.5, 726.4±0.5, 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

18. The method of claim 15, wherein one or more of said ions detected in step (d) are selected from the group consisting of ions with m/z of 636.4±0.5, 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

19. The method of claim 15, wherein said mass spectrometry comprises tandem mass spectrometry.

20. The method of claim 19, wherein said ionizing of step (c) comprises:

ionizing said peptide T129 from step (b) to generate a multiply charged peptide T129 precursor ion detectable by mass spectrometry;

and colliding said peptide T129 precursor ion with a collision gas to generate one or more peptide T129 fragment ions.

21. The method of claim 20, wherein said one or more peptide T129 fragment ions are selected from the group consisting of ions with m/z of 541.3±0.5, 612.3±0.5, 726.4±0.5, 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

22. The method of claim 20, wherein said one or more peptide T129 fragment ions are selected from the group consisting of ions with m/z of 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

23. The method of claim 20, wherein said ions detected in step (d) comprise one or more peptide T129 fragment ions.

24. The method of claim 15, wherein step (b) is accomplished by utilizing at least one size separation technique.

25. The method of claim 15, further comprising purifying thyroglobulin from a body fluid or tissue sample to generate a thyroglobulin containing test sample.

26. The method of claim 25, wherein said body fluid or tissue sample comprises plasma or serum.

27. The method of claim 15, wherein said test sample comprises a body fluid.

28. The method of claim 27, wherein said test sample comprises plasma or serum.

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